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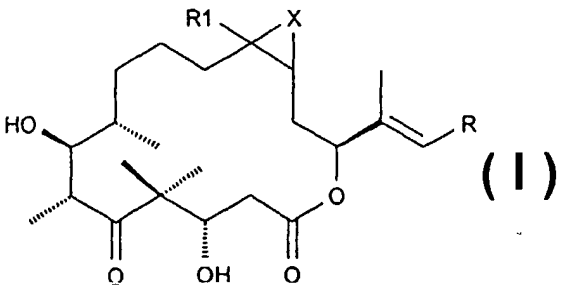
(54) Title: EPOTHILONE DERIVATIVES



(I)

(57) Abstract: This invention relates Epothilone derivatives of formula (I), wherein the symbols and substituents are as defined in the description, to processes for the preparation thereof, to pharmaceutical compositions comprising such derivatives and to the use of such derivatives , for the preparation of pharmaceutical compositions for the treatment especially of a proliferative disease, such as a tumour.

EPOTHILONE DERIVATIVES

Description

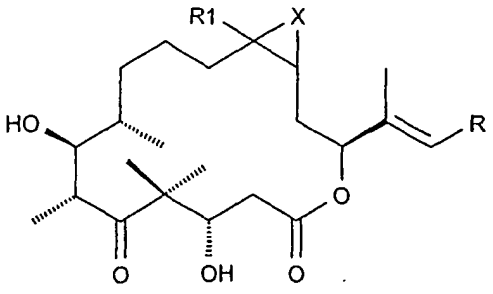
Technical Field:

The invention relates to antitumor agents. More particularly, the invention related to analogs of epothilone B, cis and trans-12,13-cyclopropyl epothilone B and cis and trans 12, 13-epoxide Epothilone B as antitumor agents.

Summary:

The invention is directed to analogs of epothilone B cis and trans-12,13-cyclopropyl epothilone B and cis and trans 12, 13-epoxide Epothilone B having potent cytotoxic active against a variety of cell lines, including Taxol®-resistant tumor cells. Exemplary embodiments of the invention include compounds **3**, **6**, and **8-14**, as illustrated in Figure 1 and compounds **3**, **49**, and **50-60**, as illustrated in figure 11. Another aspect of the invention is directed to the use of such compounds as cytotoxic agents.

One aspect of the invention is directed to a compound represented by formula I:

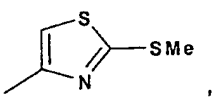


wherein,

wherein,

when a compound of formula I is a cis- isomer X is oxygen or CH_2 and when a compound of formula I is a trans- isomer X is CH_2;

when compound of formula I is a cis- isomer and X is oxygen then R1 is methyl and R is a radical selected from the group consisting of



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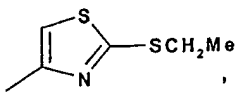

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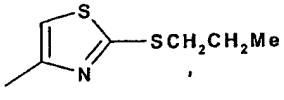

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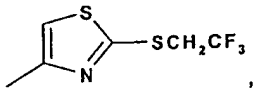

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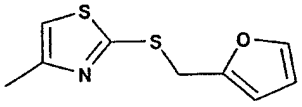

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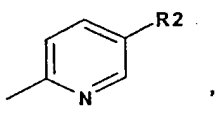

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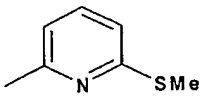

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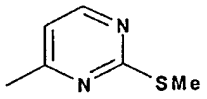
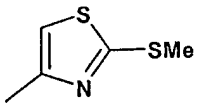


wherein R2 is a radical selected from the group consisting of -Me, -Cl, -Br, -SMe and $-CF_3$;

when a compound of formula I is a cis isomer and X is CH_2 then R1 is methyl and R is a radical selected from the group consisting of



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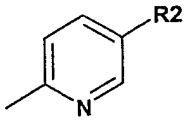
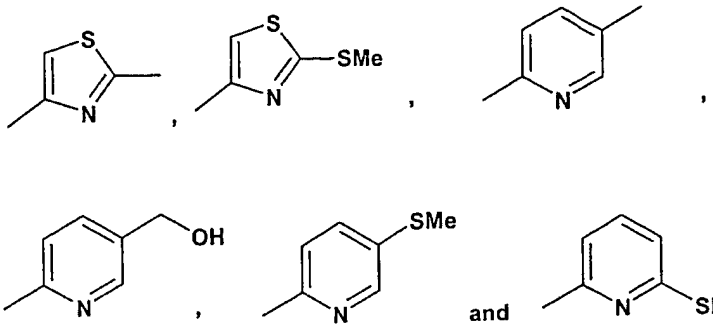


wherein R2 is a radical selected from the group consisting of -Me, -Cl, -Br, -SMe and $-CF_3$;

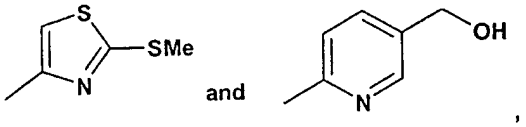
when a compound of formula I is a trans isomer and X is CH_2 then R1 is methyl or Hydrogen;

wherein, when R1 is methyl R is a radical selected from the group consisting of



and, when R1 is hydrogen,

R is a radical selected from the group consisting of



or a salt of a compound of formula I where a salt-forming group is present.

Where the plural form is used for compounds, salts, and the like, this is taken to mean also a single compound, salt, or the like ("a" as an indefinite article or as a numeral meaning "one").

Salts are primarily the pharmaceutically acceptable salts of compounds of formula I.

Such salts are formed, for example, as acid addition salts, preferably with organic or inorganic acids, from compounds of formula I with a basic nitrogen atom, especially the pharmaceutically acceptable salts. Suitable inorganic acids are, for example, hydrohalic acids, such as hydrochloric acid, sulphuric acid, or phosphoric acid.

Suitable organic acids are, for example, carboxylic, phosphonic, sulphonic or sulphamic acids, for example acetic acid, propionic acid, octanoic acid, decanoic acid, dodecanoic acid, glycolic acid, lactic acid, 2-hydroxybutyric acid, gluconic acid, glucosemonocarboxylic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, glucaric acid, galactaric acid, amino acids, such as glutamic acid, aspartic acid, N-methylglycine, acetylaminoacetic acid, N-acetylasparagine or N-acetylcysteine, pyruvic acid, aceto-acetic acid, phosphoserine, 2- or 3-glycerophosphoric acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexanecarboxylic acid, benzoic acid, salicylic acid, 1- or 3-hydroxynaphthyl-2-carboxylic acid, 3,4,5-trimethoxybenzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, glucuronic acid, galacturonic acid, methane- or ethane-sulphonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulphonic acid, benzene-sulphonic acid, 2-naphthalenesulphonic acid, 1,5-naphthalene-disulphonic acid, N-cyclohexylsulphamic acid, N-methyl-, N-ethyl- or N-propyl-sulphamic acid, or other organic protonic acids, such as ascorbic acid.

Salts of compounds of formula I with a salt-forming group may be prepared in a manner known per se. Acid addition salts of compounds of formula I may thus be obtained e.g. by treatment with an acid or with a suitable anion exchange reagent.

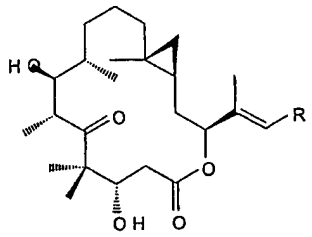
Salts can usually be converted to free compounds, e.g. by treating with suitable basic agents, for example with alkali metal carbonates, -hydrogencarbonates, or -hydroxides, typically potassium carbonate or sodium hydroxide.

For isolation or purification purposes it is also possible to use pharmaceutically unacceptable salts, for example picrates or perchlorates. Only the pharmaceutically acceptable salts or free compounds (if the occasion arises, in the form of pharmaceutical preparations) attain therapeutic use, and these are therefore preferred.

In view of the close relationship between the novel compounds in free form and in the form of their salts, including those salts that can be used as intermediates, for example in the purification or identification of the novel compounds, hereinbefore

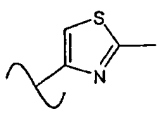
and hereinafter any reference to the free compounds is to be understood as referring also to the corresponding salts, as appropriate and expedient.

Another aspect of the invention is directed to a compound represented by formula II:



II

In Formula II, R is a radical selected from the group consisting of the following structures: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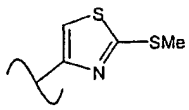
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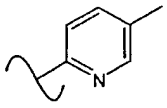
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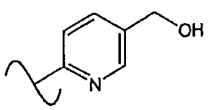
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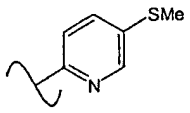
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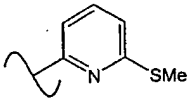
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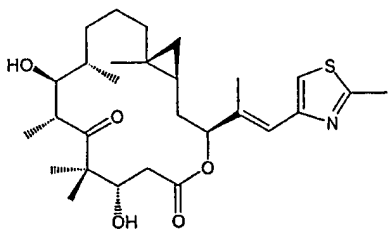
Preferred embodiments of this aspect of the invention include compounds represented by the following formulae: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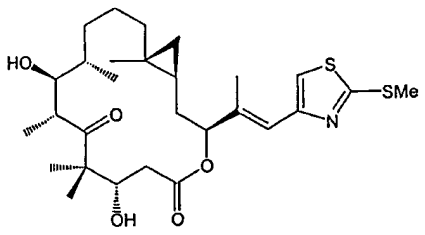
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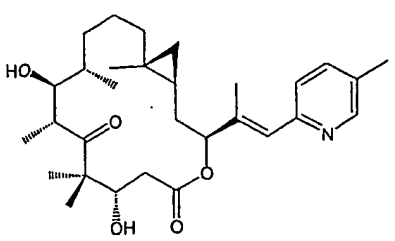
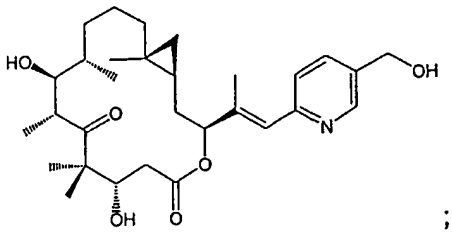
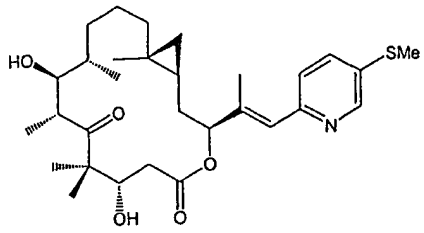
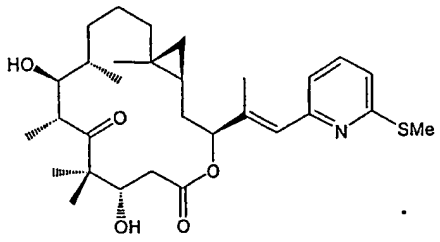
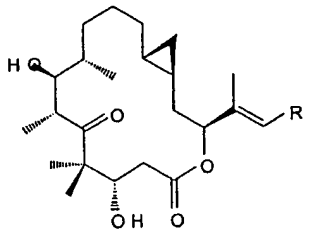





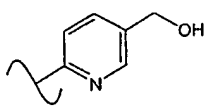
; and .

Another aspect of the invention is directed to a compound represented by formula III:



III

In formula III, R is a radical selected from the group consisting of the following structures: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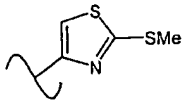
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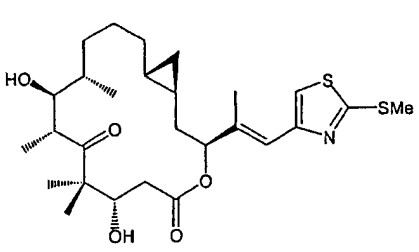
Preferred embodiments of this aspect of the invention are represented by the following formula:



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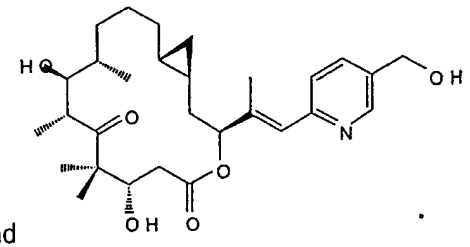
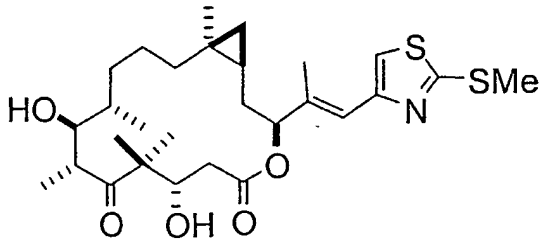
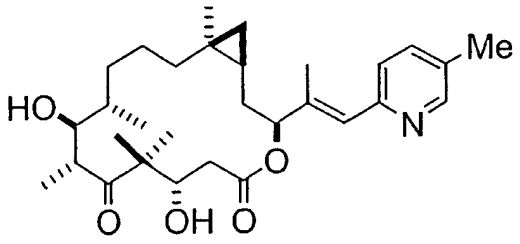


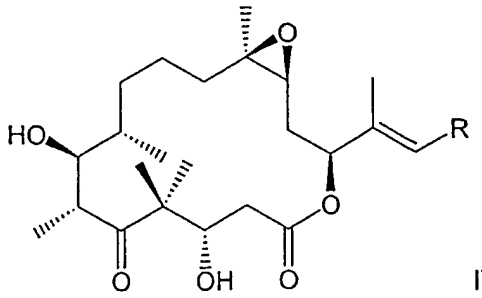
Another aspect of the invention is directed to a compound represented by the following structure:



.Another aspect of the invention is directed to a compound represented by the following structure:

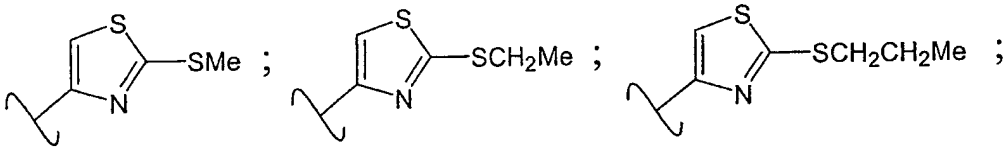


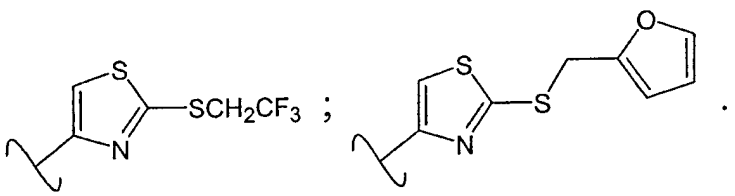
Another aspect of the invention is directed to a compound represented by formula IV:



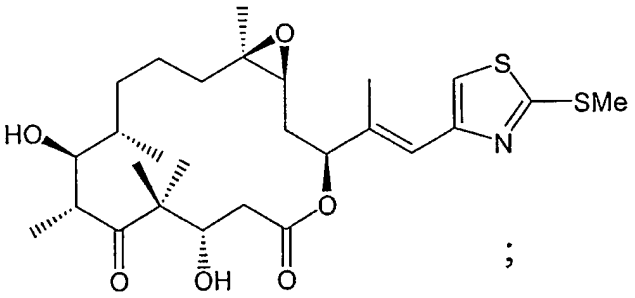
IV

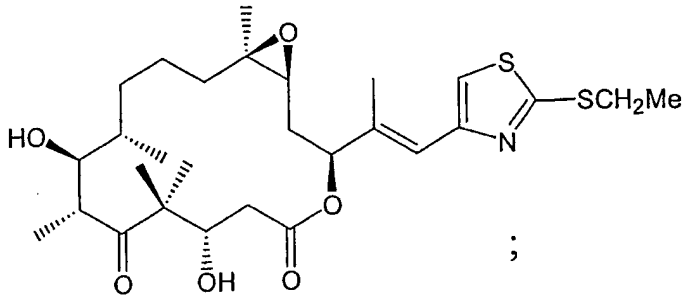
wherein R is a radical selected from the group consisting of radicals represented by the following structures:





Preferred embodiments of this aspect of the invention include compound represented by the following structures:





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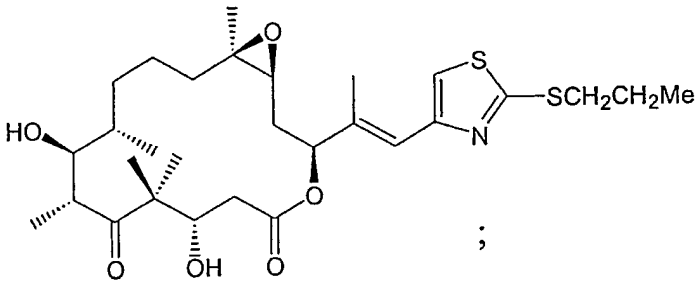

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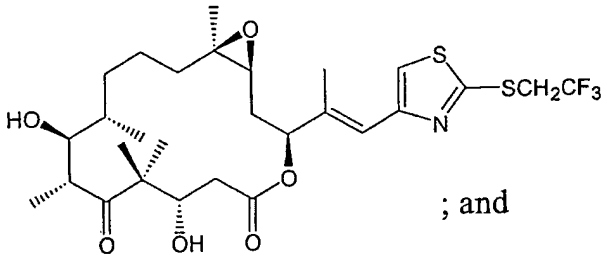
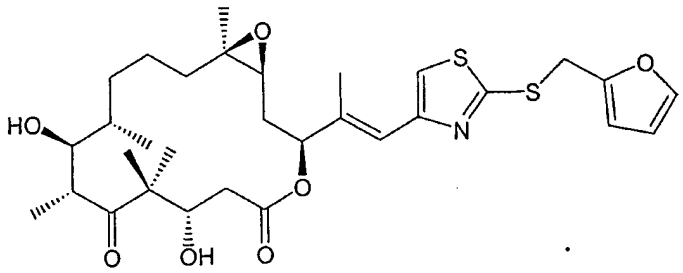
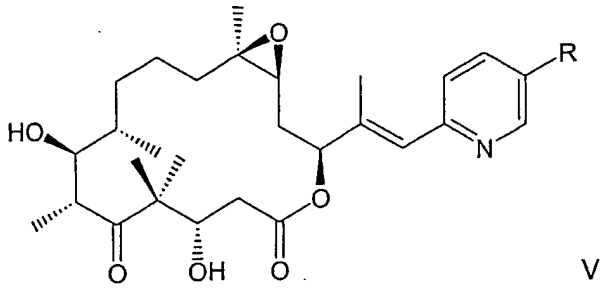


; and



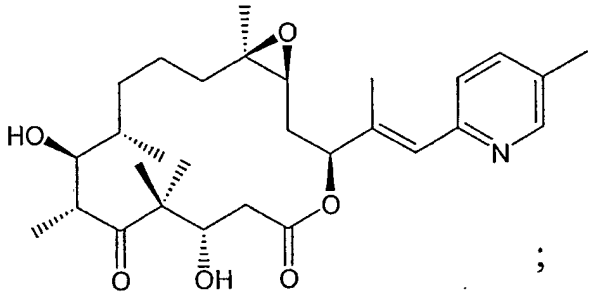
.

Another aspect of the invention is directed to a compound represented by the formula V:



V

wherein, R is a radical selected from group consisting of -Me, -Cl, -Br, -SMe, and $-CF_3$. Preferred embodiments of this aspect of the invention include compounds represented by the following structures:



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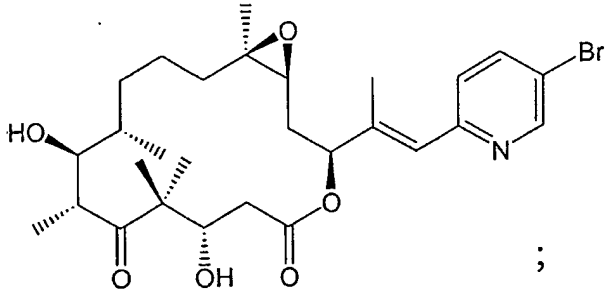
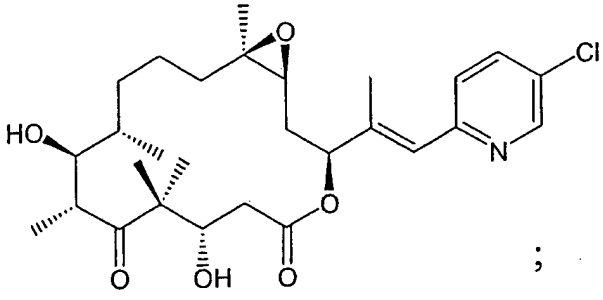
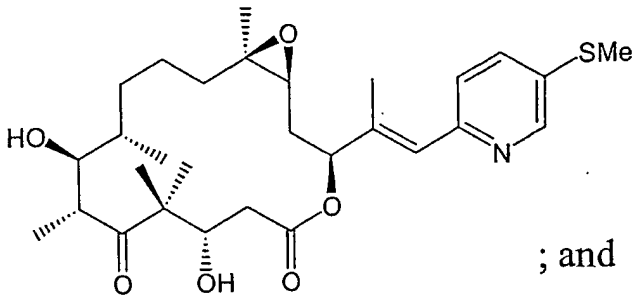


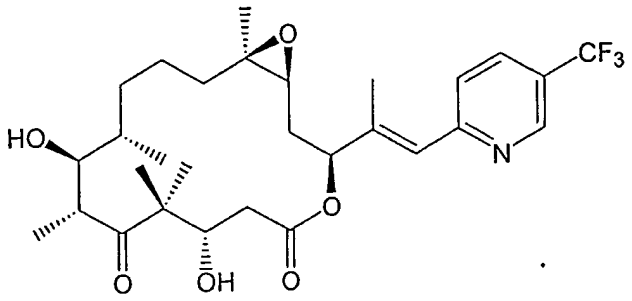
;



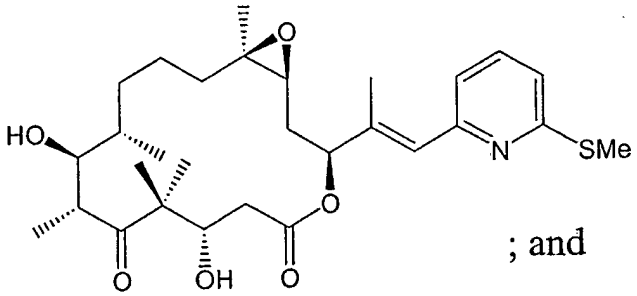
;



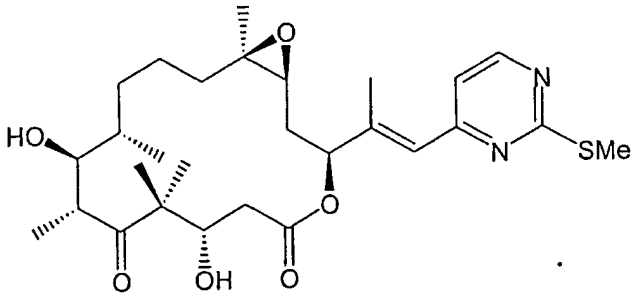
; and



Other aspects of the invention are directed to compounds represented by the following structures:



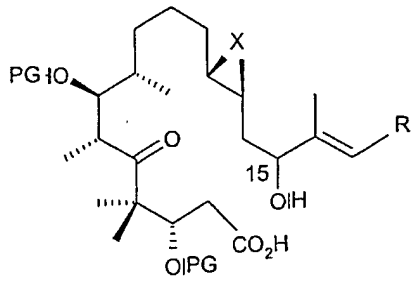
; and



Another aspect of the invention is a process for synthesizing any of the compounds described above or intermediates thereof, as described in the specification, in particular

a process for the preparation of a compound of formula I, II, III, IV or V

wherein a compound of the formula VI



(VI)

wherein X and R have any of the meanings as defined herein before and PG is a protecting group for a hydroxy function,

in a first step is condensed by a esterification reaction, optionally in the presence of a catalyst,

and in a second step the protecting group is detached thus furnishing a lacton of formula I.

The term "protecting groups for a hydroxy group" as used herein refers to acid labile protecting groups for a hydroxy group, which groups are known as such. It is a characteristic of protecting groups that they lend themselves readily, i.e. without undesired secondary reactions, to removal, typically by solvolysis, reduction, photolysis or also by enzyme activity, for example under conditions analogous to physiological conditions, and that they are not present in the end-products.The specialist knows, or can easily establish, which protecting groups are suitable with the reactions mentioned hereinabove and hereinafter.

The protection of hydroxy groups by protecting groups, the protecting groups themselves, and their cleavage reactions are described for example in standard reference works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in T. W. Greene, "Protective

Groups in Organic Synthesis", Wiley, New York 1981, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie" (*Methods of organic chemistry*), Houben Weyl, 4th edition, Volume 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, "Aminosäuren, Peptide, Proteine" (*Amino acids, peptides, proteins*), Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide und Derivate" (*Chemistry of carbohydrates: monosaccharides and derivatives*), Georg Thieme Verlag, Stuttgart 1974.

Preferred protecting groups are silyl ethers which are acid labile like *tert*-butyl-dimethyl-silyl (TBS) ether, triethylsilyl (TES) ether, triisopropylsilyl (TIPS) ether, diethylisopropylsilyl (DEIPS) ether, isopropyldimethylsilyl (IPDMS) ether or thexyldimethylsilyl (TDS) ether.

Brief Description of Drawings:

Figure 1 illustrates the structures of selected natural and designed epothilones. Grey boxes indicate compounds synthesized in this study.

Figure 2 illustrates a chart disclosing the displacement of the fluorescent taxoid Flutax-2 (50 nM) from microtubule binding sites (50 nM) by competing ligands at 37°C.

Figure 3 illustrates the synthesis of 2-(thiomethyl)thiazole epothilone B (**3**) via Stille coupling.

Figure 4 illustrates a retrosynthetic analysis of *trans*-cyclopropyl epothilone B analogues (**1 - 6, 8, 10**, and **12–14**).

Figure 5 illustrates the construction of aldehyde **32**.

Figure 6 illustrates the construction of vinyl iodides **20c–g**.

Figure 7 illustrates the synthesis of epothilone analogues **8–14**.

Figure 8 illustrates a table disclosing the cytotoxicity of epothilones **1** through **14** and paclitaxel against 1A9 human ovarian carcinoma cells and □-tubulin mutant cell lines selected with paclitaxel or epothilone A.

Figure 9 illustrates a table disclosing the tubulin polymerization potency and cytotoxicity of epothilones **1–8**, **10–14**, and paclitaxel against human epidermoid cancer cell lines.

Figure 10 illustrates a table disclosing binding affinities of epothilone analogues to the taxoid binding site of microtubules.

Figure 11 shows a series of structures of the various designed analogs of epothilones A and B along with the structures of epothilone A and B.

Figure 12 is a scheme showing the last step in the synthesis of many of the analogs from the vinyl iodide **15**.

Figure 13 is a scheme showing the steps required to synthesize the stannanes used in the scheme in Figure 12.

Figure 14 is scheme illustrating the synthetic route taken to build the skeleton of the cyclopropyl analogs of epothilone B.

Figure 15 is a scheme showing the final steps used in the synthesis of cyclopropyl analogs 48 and 50.

Figure 16 is a table with the cytotoxicities of epothilones 48, 50 and 51-60 against human carcinoma cells and β-tubulin mutant cell lines selected with paclitaxel or epothilone A.

Figure 17 is a table with the cytotoxicities (IC50's in nM) of selected epothilones against the human epidermoid cell lines KB-3 and KB-8511.

Another aspect of the invention is directed to a pharmaceutical composition containing a therapeutic dose of a compound within either formula I, II, III, IV or

formula V, represented above, for the treatment of a proliferative disease in a mammal. In a preferred mode, the mammal is a human.

Detailed Description

The construction of a series of epoxide and cyclopropane epothilones with varying side chains by chemical synthesis and biologically evaluated is disclosed. The design of the present focused epothilone library was based on the current knowledge of structure activity relationships (SAR), specifically the facts that: (1) epothilone B (**2**) is considerably more potent than epothilone A (**1**); (2) a thiomethyl replacement for the methyl group on the thiazole moiety enhances the potency (Nicolaou, K. C.; et al. *Angew. Chem.* **1998**, *110*, 2120–2153; *Angew. Chem. Int. Ed.* **1998**, *37*, 2014–2045. Nicolaou, K. C.; et al. *Tetrahedron* **2002**, *58*, 6413–6432; Nicolaou, K. C.; et al. *Angew. Chem.* **1998**, *110*, 89–92; *Angew. Chem. Int. Ed.* **1998**, *37*, 84–87.); (3) a heterocycle such as pyridine (Nicolaou, K. C.; et al. *Chem. Biol.* **2000**, *7*, 593–599.) replacement for the thiazole ring needs to maintain the proper position for the nitrogen for biological activity; and (4) a cyclopropane ring can replace the epoxide moiety without loss of activity (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313–9323; Nicolaou, K. C.; et al. *ChemBioChem.* **2001**, *2*, 69–75; Johnson, J. A.; et al. *Org. Lett.* **2000**, *2*, 1537–1540.). From these considerations, epothilones **3**, **6** and **8-14** (figure 1) and **48**, **50** and **51–60** (Figure 11) were considered as prime candidates for chemical synthesis and biological evaluation.The biological evaluation of these compounds led to the identification of the thiomethylthiazole side chain as a desirable pharmacophoric group improving the biological activity of the epothilones with regard to cytotoxicity and tubulin polymerizing properties. The enhanced activity was confirmed by three distinct biological assays where the effects of the compounds tested were determined both in cells and *in vitro*.

Design and chemical synthesis of epothilone analogues:

As an initial foray, we decided to confirm the potency enhancement bestowed on the epothilone scaffold by the methylthio group as compared to the methyl substituent in the epothilone B series. The methylthiothiazole epothilone B (**3**) was thus synthesized by Stille coupling of stannane **16** (Nicolaou, K. C.; et al. *Bioorg. Med. Chem.* **1999**, *7*, 665-697) with vinyl iodide **15** (Nicolaou, K. C.; et al. *Chem. Eur. J.* **2000**, *6*, 2783-2800) (80% yield) as shown in Figure 3. The observed high potency of analogue **3** against a series of tumor cell lines (see Table 1) encouraged us to proceed with the design and synthesis of an entire family of methylthio analogues as well as a number of new pyridine-containing epothilones.

The designed epothilone analogs (**51–60**) were synthesized in a convergent manner from vinyl iodide **15** (Nicolaou, K. C.; et al. *Chem. Eur. J.* **2000**, *6*, 2783–2800.) and the corresponding aromatic stannanes as shown in Figure 12 (for abbreviations of reagents and protective groups, see the detailed description of figures). Thus, a Stille-type coupling of **15** with appropriate stannanes (**64a–d**, **66a–d**, **67** and **68**) was carried out in the presence of $PdCl_2(MeCN)_2$, CuI and $AsPh_3$ in DMF at ambient temperature, leading directly to the desired epothilones (**51–60**) in the indicated yields. The required aromatic stannanes were prepared as summarized in Figure 13. Thus, for the thiazole compounds **64a–64d**, the commercially available 2,4-dibromothiazole (**62**) was reacted with the corresponding thiol in the presence of NaH leading first to the intermediate sulfides (**63a–63d**) through replacement of the more reactive 2-bromide substituent. Subsequent coupling of these substrates with $Me_3SnSnMe_3$ in the presence of $Pd(PPh_3)_4$ in toluene at 100 _C then gave the desired products **64a–64d** via reaction of the second bromide residue. The pyridyl stannanes **66a–66d** were similarly synthesized from the readily available 2-bromopyridines **65a**, **65b** (Virgilio, N. *J. Org. Chem.* **1973**, *38*, 2660–2664), **65c** (Nicolaou, K. C.; et al. *Tetrahedron* **2002**, *58*, 6413–6432) and **65d** (Testaferri, L.; et al. *Tetrahedron* **1985**, *41*, 1373–1384) via metal-halogen exchange (*n*BuLi) followed by quenching of the resulting 2-lithioderivatives (Gilman, H.; et al. *J. Org. Chem.* **1951**, *16*, 1788–1791) with $n Bu_3SnCl$. Stannanes **67** (Dinnell, K.; et al. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1237–1240) and **68**

(Jessie, S.; Kjell, U. *Tetrahedron* **1994**, *50*, 275–284) were prepared according to the corresponding literature procedures from the respective halides.

Figure 4 outlines, in retrosynthetic format, the pathway that was followed for the construction of the cyclopropyl epothilone B analogues. Based on our previously reported strategy, the adopted sequence required a Charette cyclopropanation reaction (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313-9323; Charette, A. B.; et al. *J. Am. Chem. Soc.* **1998**, *120*, 11943-11952) to establish early on in the synthesis the 12,13-cyclopropyl site, an aldol reaction according to our optimized procedure (Nicolaou, K. C.; et al. *Chem. Eur. J.* **2000**, *6*, 2783-2800) to construct the C6-C7 bond with its two stereocenters, a Nozaki-Hiyama-Kishi coupling (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313-9323; Takai, K.; et al. *Tetrahedron Lett.* **1983**, *24*, 5281-5284; Jin, H.; et al. *J. Am. Chem. Soc.* **1986**, *108*, 5644-5646) to introduce the side chain, and a Yamaguchi macrolactonizaion (Inanaga, J.; et al. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993; Mulzer, J.; et al. *Synthesis* **1992**, 215-228; Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **1997**, *119*, 7974-7991) to complete the macrocyclic structure. Key building blocks **18** or **69** and **19**, and **20** were thus defined as the starting points for these constructions. Construction of the corresponding epothilone A analogues was envisaged to be carried out in the same manner as previously reported by us (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313-9323).

Figure 5 and figure 14 outline the synthesis of the required aldehyde **32** or **82** from the readily available geraniol (**18** or **69**). Thus, Charette cyclopropanation of **18** (Et_2Zn-CH_2I_2, in the presence of chiral ligand **21**) (Charette, A. B.; et al. *J. Am. Chem. Soc.* **1998**, *120*, 11943-11952) furnished cyclopropyl alcohol **22** in 87% yield and 93% ee or **71** in 80% yield, 95%ee. Protection of the hydroxy group in **22** or **71** (NaH-BnBr) (for abbreviations of reagents and protecting groups, see legends in schemes) followed by ozonolysis (O_3; $NaBH_4$) of the remaining double bond led to compound **23** or **72** in 89% or 83% overall yield respectively. Conversion of alcohol **23** or **72** to the corresponding iodide (**24**, 95% yield or **73**, 91%) was accomplished upon mesylation and subsequent reaction with NaI. Alkylation of (–)-

propionaldehyde SAMP hydrazone (**25**) (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **1997**, *119*, 7974-7991; Enders, D. *Aymmetric Synth.* **1984**, *3*, 275-339; Enders, D.; Klatt, M. *Synthesis* **1996**, 1403-1418) with iodide **24** or **73** under the influence of LDA gave compound **26** or **75** (84% yield, 87% yield respectively), whose cleavage (MeI; HCl_{aq}) led to aldehyde **17** in 86% yield or **76** in 91% yield. The ratio of the resulting C-8 epimers was determined to be *ca.* 97:3 by ^1H NMR analysis of the MTPA esters derived from aldehyde **17** (Tsuda, M.; Endo, T.; Kobayashi, J. *J. Org. Chem.* **2000**, *65*, 1349-1352 and references cited therein). The aldol condensation between ketone **19** and aldehyde **17** or **76** under the previously defined conditions [LDA (2.4 equiv), ketone **19** (2.3 equiv), –78 to –40 °C, 30 min; then aldehyde **17** or **76**, –78 °C, 5 min] (Nicolaou, K. C.; et al. *Chem. Eur. J.* **2000**, *6*, 2783-2800) afforded aldol product **27** or **78** which was isolated in a diastereomerically pure form (81% yield). Subsequent protection of the secondary alcohol in **27** or **78** as a TBS ether (TBSOTf, 2,6-lutidine) followed by selective cleavage of the primary TBS group (HF•py) afforded, in 88% overall yield, alcohol **28** or 86% overall yield, alcohol **79**. The compound was stepwise oxidized to the carboxylic acid (DMP; then $NaClO_2$) which was then protected as the TMSE ester **29** or **80**(TMSE-OH, EDC, 4-DMAP) in 75% or 73% overall yield. Hydrogenolysis of the benzyl ether in **29** or **80** followed by oxidation with DMP led to aldehyde **30** or **82** (84% yield, 87% yield) whose homologation (NaHMDS-$MeOCH_2PPh_3Cl$; then PPTS) to the coveted higher aldehyde **32** or **83** proceeded smoothly, and via vinyl ether **31** (*ca.* 1:1 *E*:*Z* ratio), with 82% overall yield.

The side chains (**20a–g**, Scheme 4 and **64a-d**, **66a-d**, **84a** and **84b**, scheme 15) were synthesized either as previously reported (**20a**, **20b** and **84a**) (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313-9323), (**84b**) (Nicolaou, K. C.; et al. *J. Tetrahedron* **2002**, *58*, 6413-6432), or from the corresponding aryl halides (**33** (Ellingboe, J. W.; et al. *J. Med. Chem.* **1994**, *37*, 542-550), **37**, **38**, **39**) as shown in Scheme 4. Protection of 4-hydroxymethyl-2-pyridyl bromide **33** as a trityl ether (TrCl, 4-DMAP, 100%) followed by Sonogashira coupling (Arcadi, A.; et al. *Tetrahedron* **1994**, *50*, 437-452) of the resulting aryl bromide **34** with propyne [$Pd(PPh_3)_2Cl_2$-CuI,

96%] led to acetylenic compound **35** which served as a precursor to vinyl iodide **20c** (*n*-BuLi; then $(n\text{-}Bu_3Sn)_2$, CuCN, MeOH; then I_2, 80% yield). Exchange of the trityl for a MOM group within **35** [HCl(g), $CHCl_3$; then NaH, MOM-Cl, 34% overall yield] (Betzer, J.-F.; et al. *Tetrahedron Lett.* **1997**, *38*, 2279-2282) allowed access to vinyl iodide **20d** (67% yield) by exposure of the resulting intermediate **36** to the same conditions described above for the **35** to **20c** conversion. Similar chemistry was employed to construct vinyl iodides **20e–20g** from **37–39**, respectively, as shown in Scheme 4.

Two crucial bond formations and two accompanying deprotections separated key building blocks **32** (prepared in this study for epothilone B analogues), **40** (prepared as previously described for epothilone A analogues) (Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313-9323), and **20a–g** (for side chains) from the targeted epothilone analogues. The first operation was the Nozaki-Hiyama-Kishi coupling (Takai, K.; et al. *Tetrahedron Lett.* **1983**, *24*, 5281-5284; Jin, H.; et al. *J. Am. Chem. Soc.* **1986**, *108*, 5644-5646) of aldehydes **32** and **40** with vinyl iodides **20a–g**. This carbon-carbon bond forming reaction worked admirably in this instance ($CrCl_2$, $NiCl_2$, 4-*t*-BuPy, DMSO), furnishing, after TBAF-induced carboxylic acid generation, coupling products (**41a, 41b, 41d–g, 42c, 42e, 85 and 88**) in yields indicated in Schemes 5 and 15 (as *ca.* 1:1 mixtures of C-15 diastereomers). Each mixture of hydroxy acid diastereomers (**41a, 41b, 41d–g, 42c, 42e, 85 and 88**) was then subjected to Yamaguchi macrocyclization (2,4,6-trichlorobenzoyl chloride, 4-DMAP) (Inanaga, J.; et al. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989–1993; Mulzer, J.; et al. *Synthesis* **1992**, 215–228) to afford the desired 15(*S*) lactone in the indicated (unoptimized) yields together with its 15(*R*) epimer. The separation of the two epimers at this juncture was facilitated by their rather drastically different R_f values on silica gel. Final deprotection of protected derivatives either with 20% TFA in CH_2Cl_2 (**43a, 43b, 43e–g, 44c, 44e, 86 and 89**) or with TMSBr-4Å MS in CH_2Cl_2, followed by 20% TFA in CH_2Cl_2 (**43d**), led to epothilones **6, 8–14** in the indicated (unoptimized) yields (Schemes 5 and 15). Chromatographically and spectroscopically pure compounds were subjected to biological evaluations as described below.

Chemical biology:

The biological activities of the synthesized epothilones were evaluated through cytotoxicity, *in vitro* tubulin polymerization, and tubulin binding assays. Cytotoxicity was first evaluated in a set of ovarian carcinoma cell lines, including a parental cell line (IA9) and three drug-resistant cell lines, namely the paclitaxel-resistant strains (Giannakakou, P.; et al. *J. Biol. Chem.* **1997**, *272*, 17118-17125) IA9/PTX10 and IA9/PTX22 and the epothilone-resistant strain (Giannakakou, P.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2904-2909) 1A9/A8. These resistant cell lines harbor distinct acquired β-tubulin mutations which affect drug-tubulin interaction and result in impaired taxane and epothilone-driven tubulin polymerization. The results of these biological investigations are summarized in Table 1 (Skehan, P.; et al. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112). Further cytotoxicity and *in vitro* tubulin polymerization assays were carried out using a set of human epidermoid cancer cell lines, including a parent cell line (KB-31) and a paclitaxel-resistant (due to Pgp overexpression) cell line (KB-8511). The results of these studies are summarized in Table 2 (Nicolaou, K. C.; et al. *Chem. Biol.* **2000**, *7*, 593-599; Meyer, T.; et al. *Int. J. Cancer* **1989**, *43*, 851-856).

In general, there is good agreement between the *in vitro* tubulin polymerization potency and the cytotoxicity profile of the tested compounds against both the 1A9 human ovarian carcinoma cells and the KB-31 human epidermoid carcinoma cells. In agreement with original observations with the naturally occurring epothilones A and B, none of the epothilone A or B analogues tested herein appears to be a good substrate for the drug-efflux pump P-glycoprotein (Pgp). This is evident by the lack of cross-resistance of each of these analogues to the Pgp expressing cell line KB-8511, in contrast to paclitaxel-a known Pgp substrate- which is 214-fold less active against KB-8511 cells (see figure 9 and figure 17). It is noteworthy that all the epothilone analogues appear more active against the β-tubulin mutants compared to epothilone A (**1**) and epothilone B (**2**) (see figure 8 and figure 16, RR values). This is more pronounced with compounds **10–14, 52, 54, 58 and 60** for which the relative

resistance values (RR) range from 1.6–9.3 against PTX10 (β270) and A8 (β274) cells compared with 9.4-24.9 RR values for Epo A (**1**) and Epo B (**2**) (figure 8). Furthermore, in the current study, and in agreement with previous reports (Nicolaou, K. C.; et al. *ChemBioChem* **2001**, 2, 69-75; Giannakakou, P.; et al. *J. Biol. Chem.* **1997**, 272, 17118-17125; Giannakakou, P.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, 97, 2904-2909), we found that the paclitaxel-selected mutant PTX22 (β364) retains almost full sensitivity to the epothilones, and to all epothilone analogues tested in this report (RR values æ 3.3).

There is a general agreement in the relative potency of the substituted epothilone B analogs against the 1A9 human ovarian and the KB-31 human epidermoid cancer cells. Collectively, the results of these cytotoxicity assays revealed interesting information in terms of structure-activity relationships within the epothilone family. First, compounds 4 and 6 in which the C12-C13 epoxide moiety is replaced by a cyclopropane ring are the two most potent compounds among all the epothilone B analogs presented here. This result reaffirms that the C12-C13 epoxide moiety is not necessary for biological activity as previously noted (Nicolaou, K. C.; et al. J. Am. Chem. Soc. 2001, 123, 9313–9323; Nicolaou, K. C.; et al. ChemBioChem. 2001, 2, 69–75; Johnson, J. A.; et al. Org. Lett. 2000, 2, 1537–1540.). Compound 4 is 6-fold more active than the parent epothilone B (2) against the 1A9 human ovarian carcinoma cells (Figure 6) further confirming that the replacement of the methyl group on the thiazole side-chain with a thiomethyl group leads to increased activity. This result is in agreement with previous data on a similar substitution in epothilone B without replacement of the C12-C13 epoxide (i.e. compound 3) (Nicolaou, K. C.; et al. Tetrahedron 2002, 58, 6413–6432). The latter compound (3) was about 2-fold more active than the parent epothilone B, while compound 4 is 6-fold more potent than epothilone B. This result makes compound 4, the most active epothilone B analog against the 1A9 cell line synthesized to date and suggests that replacement of the epoxide by a cyclopropane moiety together with the replacement of the methyl substituent on the thiazole moiety with a thiomethyl group act synergistically, leading to the observed enhancement of biological activity. Interestingly, substitution of the methyl group of the thiazole ring with larger moieties

(compounds 7–10) (The IC50 value for compound 7 was found to be 2.5 nM against the 1A9 cell line.) led to diminished biological activity as compared to epothilone B (Figures 6 and 7).

Among the epothilone B analogs with substituted pyridine side-chains at the 5-position of the pyridine ring (compounds 55–57 and 59), the thiomethyl analog (compound 57) is the most potent followed by the bromo-substituted derivative (compound 55) followed by the chloro-substituted system (compound 56). When the thiomethyl group is relocated from the 5-position of the pyridine ring (compound 57) to the 6-position (compound 58) loss of activity occurs as the IC50 value drops from 0.4 nM (compound 57) to 3.3 nM (compound 58) (Figure 16). Furthermore, replacement of the thiomethyl group at the 5-position of the pyridine ring (compound 57) with a trifluoromethyl group (compound 59) results in loss of activity by 10-fold. Finally, the least active of the synthesized epothilone B analogs is compound 60 where a pyrimidine side-chain with a thiomethyl substitution has replaced the thiazole side-chain of the parent compound.

In addition to the above biological assays, the relative potency of each epothilone analogue was measured by the fluorescent taxoid displacement assay (Andreu, J. M.; Barasoain, I. Biochemistry 2001, 40, 11975-11984). The purpose of these experiments was to compare the equilibrium constants with which microtubules bind at their taxane site the epothilone analogues investigated. The inhibition of the binding of the well-characterized fluorescent taxoid Flutax-2 (Souto, A. A.; et al. Angew. Chem. Int. Ed. Engl. 1995, 34, 2710-2712; Díaz, J. F.; et al. J. Biol. Chem. 2000, 275, 26265-26276; Abal, M.; et al. Cell. Motil. Cytoskeleton 2001, 49, 1-15) to microtubules by each of the epothilone analogues was measured at 37 °C (Figure 2). The resulting equilibrium dissociation constants shown in Table 3 indicate that epothilone A (1) has the lowest binding affinity among the epothilone analogues tested (Kd = 34Å4). The most powerful ligand among those measured in this assay is compound 3, with a Kd value of 0.64Å0.24 nM, followed by compounds 8, 11–13, with similar Kd values comprised between 1.6 and 1.9 nM. With the possible exception of compound **13**, the binding affinities of the analogues tested mirror their

respective activities in both cell growth inhibition and *in vitro* tubulin polymerization assays.

Collectively from all three biological assays employed herein, a number of conclusions can be drawn in terms of structure-activity relationships within the epothilone family. First, the addition of the C12 methyl group does not enhance the activity in the *trans*-cyclopropyl series (compound **5** *vs* **6**, **7** *vs* **8**, **9** *vs* **10**), contrary to the result in the *cis* epoxide series, where epothilone B (**2**) is at least 10-fold more active than epothilone A (**1**). This could be due to the different orientation of the C12 methyl group in the *cis* and *trans* compounds or to overall differences in conformation between the *cis* and *trans* compounds, although the details remain to be elucidated. Second, the introduction of the 2-thiomethylthiazole side chain enhances the activity compared with the natural 2-methylthiazole side chain (compounds **2** *vs* **3**, **5** *vs* **11**, and **6** *vs* **12**). This effect was previously observed for epothilone C and D analogues (Nicolaou, K. C.; et al. *Angew. Chem.* **1997**, *109*, 2181-2187; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097-2103; see also: Sinha, S. C.; et al. *ChemBioChem* **2001**, *2*, 656-665). Third, the replacement of a methyl group with a thiomethyl group in the pyridine side chain series (compounds **8** *vs* **13**) reduces potency, contrary to the results obtained for the thiazole side chains above. This conclusion was based on the cell cytotoxicity and *in vitro* tubulin polymerization data, while in the fluorescent taxoid displacement assay the replacement of the methyl group with a thiomethyl moiety in the pyridine side chain is indifferent in terms of binding affinity. This discrepancy may simply reflect differences in cell uptake and permeability of the compounds tested or differences in the sensitivity of the two tubulin assays. Despite this discrepancy, it is clear from these data that the introduction of a thiomethyl group at the thiazole side chain is a more favorable modification than the introduction of a thiomethyl group at the pyridine side chain, which may be due to differing steric requirements by the two side chain scaffolds. In agreement with previous data obtained with *cis* pyridine epothilone analogues (Nicolaou, K. C.; et al. *Chem. Biol.* **2000**, *7*, 593-599), relocation of the thiomethyl group of the pyridine side chain from the position 5 (compound **13**) to position 6 (compound **14**) resulted in significant loss of activity. Fourth, mixed results are obtained with compounds **7** *vs* **9** and **8** *vs* **10** in which the 5-methylpyridine side chain (compounds **7** and **8**) is substituted by the 5-hydroxymethylpyridine side chain (compounds **9** and **10**). This substitution appears indifferent in cytotoxicity assays against the 1A9 human ovarian carcinoma cells (Table 1) where very similar IC_{50} values are obtained for each pair (e.g. 0.6 and 0.7 nM for compounds

7 and **9**, respectively; 1.7 nM for compounds **8** and **10**). On the other hand, in the human epidermoid carcinoma cells KB-31, compound **10** is 2-fold more active than its counterpart compound **8** with IC_{50}s at 0.44 *vs* 0.9 nM, respectively. Given the small differences in the growth rate of the two human cancer cell lines that could account for the differential results, we could conclude that the introduction of the 5-hydroxymethylpyridine side chain is not likely to enhance activity in, at least, *trans*-12,13-cyclopropyl analogues of the epothilone family.

Owing to these properties, the compounds are suitable for the treatment of proliferative diseases, especially tumour diseases, including metastases; for example solid tumours such as lung tumours, breast tumours, colorectal tumours, prostate tumours, melanomas, brain tumours, pancreas tumours, neck tumours, bladder tumours, neuroblastomas, throat tumours, but also proliferative diseases of blood cells, such as leukaemia; also for the treatment of other diseases which respond to treatment with microtubule depolymerisation inhibitors, such as psoriasis.

A compound of formula I can be administered alone or in combination with one or more other therapeutic agents, possible combination therapy taking the form of fixed combinations or the administration of a compound of the invention and one or more other therapeutic agents being staggered or given independently of one another, or the combined administration of fixed combinations and one or more other therapeutic agents. A compound of formula I can besides or in addition be administered for tumour therapy in combination with chemotherapy, radiotherapy, immunotherapy, surgical intervention, or a combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above. Other possible treatments are therapy to maintain the patient's status after tumour regression, or even chemopreventive therapy, for example in patients at risk.

Therapeutic agents for possible combination are especially one or more antiproliferative, cytostatic or cytotoxic compounds, for example one or more chemotherapeutic agent(s) selected from the group comprising the classical chemotherapeutic agents, an inhibitor of polyamine biosynthesis, an inhibitor of protein kinase, especially of serine/threonine protein kinase, such as protein kinase

C, or of tyrosine protein kinase, such as epidermal growth factor receptor protein tyrosine kinase, a cytokine, a negative growth regulator, such as TGF-ß or IFN-ß, an aromatase inhibitor, and a classical cytostatic.

Compounds according to the invention are not only for the (prophylactic and preferably therapeutic) treatment of humans, but also for the treatment of other warm-blooded animals, for example of commercially useful animals, for example rodents, such as mice, rabbits or rats, or guinea-pigs. They may also be used as a reference standard in the test systems described above to permit a comparison with other compounds.

A compound of formula I may also be used for diagnostic purposes, for example with tumours that have been obtained from warm-blooded animal "hosts", especially humans, and implanted into mice to test them for decreases in growth after treatment with such a compound, in order to investigate their sensitivity to the said compound and thus to improve the detection and determination of possible therapeutic methods for neoplastic diseases in the original host.

Stereoisomeric mixtures, e.g. mixtures of diastereoisomers, can be separated into their corresponding isomers in a manner known per se by means of suitable separation methods. Diastereoisomeric mixtures may thus be separated into their individual diastereoisomers by means of fractionated crystallization, chromatography, solvent distribution, and similar procedures. This separation may take place either at the stage of one of the starting compounds or in a compound of formula I itself. Enantiomers may be separated through the formation of diastereoisomeric salts, for example by salt formation with an enantiomer-pure chiral acid, or by means of chromatography, for example by HPLC, using chromatographic substrates with chiral ligands. (Enantiomer separation is normally effected at the intermediate stage).

Pharmaceutical preparations, methods, and uses

The present invention relates also to pharmaceutical preparations that contain a compound of formula I as active ingredient and that can be used especially in the treatment of the diseases mentioned above. Preparations for enteral administration,

such as nasal, buccal, rectal or, especially, oral administration, and for parenteral administration, such as intravenous, intramuscular or subcutaneous administration, to warm-blooded animals, especially humans, are especially preferred. The preparations contain the active ingredient alone or, preferably, together with a pharmaceutically acceptable carrier. The dosage of the active ingredient depends upon the disease to be treated and upon the species, its age, weight, and individual condition, the individual pharmacokinetic data, and the mode of administration.

The precise dosage of the compounds of formula I to be employed for inhibiting proliferative disease, preferably tumors, depends upon several factors including the host, the nature and the severity of the condition being treated, the mode of administration and the particular compound employed. However, in general, satisfactory inhibition of tumors is achieved when a compound of formula I is administered parenterally, e.g., intraperitoneally, intravenously, intramuscularly, subcutaneously, intratumorally, or rectally, or enterally, e.g., orally, preferably intravenously or orally, more preferably intravenously at a single dosage of 1-300 mg/kg body weight per cycle (cycle = 3-6 weeks) or, for most larger primates, a single dosage of 50-5000 mg per treatment cycle. A preferred intravenous single dosage per 3-6 week treatment cycle is 1-75 mg/kg body weight or, for most larger primates, a daily dosage of 50-1500 mg. A typical intravenous dosage is 45 mg/kg, once every three weeks.

Usually, a small dose is administered initially and the dosage is gradually increased until the optimal dosage for the host under treatment is determined. The upper limit of dosage is that imposed by side effects and can be determined by trial for the host being treated.

The invention relates also to pharmaceutical preparations for use in a method for the prophylactic or especially therapeutic treatment of the human or animal body, to a process for the preparation thereof (especially in the form of compositions for the treatment of tumours) and to a method of treating the above-mentioned diseases, primarily neoplastic diseases, especially those mentioned above.

The invention relates also to processes and to the use of compounds of formula I for the preparation of pharmaceutical preparations which contain compounds of formula I as active component (active ingredient).

Preference is given to a pharmaceutical composition that is suitable for administration to a warm-blooded animal, especially a human or commercially useful mammal, suffering from a disease that is responsive to the inhibition of microtubule depolymerisation, for example psoriasis or especially a neoplastic disease, comprising a correspondingly effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof when salt-forming groups are present, together with at least one pharmaceutically acceptable carrier.

A pharmaceutical composition for the prophylactic or especially therapeutic treatment of neoplastic and other proliferative diseases of a warm-blooded animal, especially a human or a commercially useful mammal requiring such treatment, especially suffering from such a disease, comprising a new compound of formula I, or a pharmaceutically acceptable salt thereof, as active ingredient in a quantity that is prophylactically or especially therapeutically active against said diseases, is likewise preferred.

Pharmaceutical preparations contain from about 0.000001 % to 95 % of the active ingredient, whereby single-dose forms of administration preferably have from approximately 0.00001 % to 90 % and multiple-dose forms of administration preferably have from approximately 0.0001 to 0.5 % in the case of preparations for parenteral administration or 1 % to 20 % active ingredient in the case of preparations for enteral administration. Unit dose forms are, for example, coated and uncoated tablets, ampoules, vials, suppositories or capsules. Further dosage forms are, for example, ointments, creams, pastes, foams, tinctures, lipsticks, drops, sprays, dispersions, etc. Examples are capsules containing from about 0.0002 g to about 1.0 g active ingredient.

The pharmaceutical preparations of the present invention are prepared in a manner known per se, for example by means of conventional mixing, granulating, coating, dissolving or lyophilising processes.

The formulations suitable for parenteral administration are primarily aqueous solutions ([or example in physiological saline, obtainable by diluting solutions in polyethylene glycol, such as polyethylene glycol (PEG) 300 or PEG 400] of an active ingredient in water-soluble form, e.g. a water-soluble salt, or aqueous injectable suspensions containing viscosity-increasing agents, e.g. sodium carboxymethyl cellulose, sorbitol and/or dextran, and where appropriate stabilisers. The active ingredient, if need be together with excipients, can also be in the form of a lyophilisate and can be made into a solution before parenteral administration by the addition of suitable solvents.

Solutions such as those used, for example, for parenteral administration can also be employed as infusion solutions.

The invention similarly relates to a process or a method for the treatment of one of the above-mentioned pathological conditions, especially a disease which responds to an inhibition of microtubule depolymerisation, especially a corresponding neoplastic disease. A compound of formula I can be administered as such or in the form of pharmaceutical compositions, prophylactically or therapeutically, preferably in an amount effective against the said diseases, to a warm-blooded animal, for example a human, requiring such treatment, the compounds especially being used in the form of pharmaceutical compositions. In the case of an individual having a bodyweight of about 70 kg the dose administered is from approximately 0.1 mg to approximately 1 g, preferably from approximately 0.5 mg to approximately 200 mg, of a compound of the present invention. Administration is preferably effected e.g. every 1 to 4 weeks, for example weekly, every two weeks, every three weeks or every 4 weeks.

The present invention also relates in particular to the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, especially a compound of formula I named as a preferred compound, or a pharmaceutically acceptable salt thereof, as such or in the form of a pharmaceutical formulation containing at least one pharmaceutically employable carrier, for the therapeutical and also prophylactic treatment of one or more of the above diseases.

The present invention also relates in particular to the use of a compound of formula I, or a pharmaceutically acceptable salt thereof, especially a compound of formula I named as a preferred compound, or a pharmaceutically acceptable salt thereof, for the preparation of a pharmaceutical formulation for the therapeutical and also prophylactic treatment of one or more of the above diseases.

Experimental

General

All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Anhydrous solvents were obtained by passing them through commercially available activated alumina columns. All reagents were purchased at highest commercial quality and used without further purification. Reactions were generally monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254). E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25, 0.50 or 1 mm E. Merck silica gel plates (60F-254). Melting points (mp) are uncorrected and were recorded on a Thomas-Hoover Unimelt capillary melting point apparatus. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on Bruker DRX-600, DRX-500, AMX-400 or AC-250 instruments and calibrated using residual undeuterated solvents as an internal reference. All labeling of carbon atoms, *e.g.* C15, refers to epothilone A (**1**) numbering (see Figure 1). IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. High resolution mass spectra were recorded on a PerSeptive Biosystems Voyager™ IonSpec mass spectrometer (MALDI-FTMS) or on an API 100 Perkin-Elmer mass spectrometer (ESI).

Synthesis of epothilone 3

Stille coupling of vinyl iodide 15 with stannane 16.

A solution of $Pd_2(dba)_3(CHCl_3$ (3.9 mg, 3.8 µmol), $AsPh_3$ (4.6 mg, 15 µmol), and CuI (7.2 mg, 38 µmol) in DMF (degassed, 0.5 mL) was added at 25 °C to a solution of iodide **15** (10 mg, 19 µmol) (Nicolaou, K. C., et al., *Chem. Eur. J.* **2000**, *6*, 2783-2800) and stannane **16** (11 mg, 38 µmol) (Nicolaou, K. C., et al., *Bioorg. Med. Chem.* **1999**, *7*, 665-697) in DMF (degassed, 0.5 mL), and the resulting solution was stirred for 2 hours. Water (10 mL) was added, and the mixture was extracted with EtOAc (3 (10 mL). The combined organic phase was washed with water (30 mL), brine (30 mL), and dried (Na_2SO_4). After evaporation of the volatiles, the residue was purified by flash column chromatography (sillica, hexanes:EtOAc 2:1 (1:1) to yield epothilone **3** as a white solid (7.2 mg, 72%); TLC R_f = 0.29 (silica, hexanes:EtOAc 1:1); $[\alpha]_D$22 -53 (*c* 0.51, CH_2Cl_2); IR (film) ν_{max} 3472 (br), 2967, 2920, 1731, 1684, 1461, 1420, 1378, 1249, 1143, 1032, 973, 879, 732, 667 cm^{-1}; MALDI-FTMS *m/z* 562.2267 (MNa^+), calcd for $C_{27}H_{41}NO_6S_2Na$ 562.2267.

Construction of aldehyde 32

Alcohol 23. To a solution of cyclopropyl alcohol **22** (4.08 g, 24 mmol) (Charette, A. B.; et al. *J. Am. Chem. Soc.* **1998**, *120*, 11943-11952) in DMF (40 mL) was added sodium hydride (1.45 g, 36 mmol, 60% in mineral oil) portionwise with stirring at 0 °C. After stirring for 0.5 h at 25 °C, the mixture was cooled to 0 °C, benzyl bromide (4.3 mL, 36 mmol) was added over 2 min, and stirring was continued for 12 h at 25 °C The reaction was quenched with NH_4Cl (sat., 50 mL), the mixture was extracted with EtOAc (3 (50 mL) and the combined extract was washed with brine (2 (100 mL), dried (Na_2SO_4) and evaporated. The residue was dissolved in CH_2Cl_2:MeOH 4:1 (60 mL), and the solution was ozonized (100 L/h, *ca.* 5 g O_3/h) at –78 °C for 21 min. (***NOTE:*** *Longer reaction times must be avoided to prevent oxidation of the benzyl ether to the corresponding benzoate.*) Excess ozone was removed by flushing with N_2 for 1 min, and then $NaBH_4$ (2.75 g, 73 mmol) was added in small portions (***CAUTION!*** *Exothermic!*) followed by methanol (20 mL). The mixture was warmed to 25 °C over 1 hour, and the reaction was quenched by the addition of NH_4Cl (sat., 20 mL). The mixture was extracted with CH_2Cl_2 (2 (50 mL), and the combined extract was washed with brine (100 mL), dried (Na_2SO_4) and evaporated. The residue was

purified by flash chromatography (silica, hexanes:EtOAc 5:2) to yield **23** as a yellow oil (5.07 g, 89%). TLC R_f = 0.20 (silica, hexanes:EtOAc 3:1); $[\alpha]_D22$ –7.5 (*c* 1.76, $CHCl_3$); IR (film) ν_{max} 3390 (br), 2933, 2859, 1452, 1070, 739, 698 cm^{-1}; MALDI-FTMS *m/z* 257.1519 (MNa^+), calcd for $C_{15}H_{22}O_2Na$ 257.1512.

Iodide 24. To a solution of cyclopropyl alcohol **23** (10.08 g, 43.0 mmol) in dry CH_2Cl_2 (100 mL) at 0 °C was added methanesulfonyl chloride (4.2 mL, 54 mmol) followed by triethylamine (9.0 mL, 65 mmol) dropwise. A white precipitate started to form immediately. The mixture was stirred at 25 °C for 1 hour, then NH_4Cl (sat., 50 mL) and water (50 mL) were added and the phases were separated. The aqueous phase was extracted with EtOAc (100 mL), and the combined organic phase was washed with brine, dried (Na_2SO_4) and evaporated. The residue was dissolved in dry acetone (200 mL), and sodium iodide (19.3 g, 129 mmol) was added. The initially almost clear solution was refluxed for 40 min, during which time a white precipitate formed. Water (100 mL) was added and the mixture was extracted with ether (500 + 250 mL). The combined extract was dried and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 5:1) to yield **24** as a colorless oil (14.16 g, 95%). TLC R_f = 0.66 (silica, hexanes:EtOAc 5:1); $[\alpha]_D22$ –16 (*c* 2.05, $CHCl_3$); IR (film) ν_{max} 2916, 2848, 1453, 1217, 1098, 1073, 735, 697 cm^{-1}; ESI-MS *m/z* 367 (MNa^+), calcd for $C_{15}H_{21}IONa$ 367.

Hydrazone 26. A solution of LDA was prepared by adding *n*-BuLi (13.1 mL, 21.0 mmol, 1.6 M in hexanes) to diisopropylamine (2.94 mL, 21.0 mmol) in THF (10 mL) at –78 °C, then warming the solution to 0 °C, and stirring for 10 min. To this LDA solution was added propionaldehyde SAMP hydrazone **25** (3.32 g, 19.5 mmol) (Nicolaou, K. C., et al., *J. Am. Chem. Soc.* **1997**, *119*, 7974-7991; Enders, D. *Aymmetric Synth.* **1984**, *3*, 275-339; and Enders, D., et al., *Synthesis* **1996**, 1403-1418), and the mixture was stirred for 6 h at 0 °C, during which time a white precipitate formed. The mixture was cooled to –98 °C (MeOH/N_2(l) bath) and a solution of iodide **24** (5.16 g, 15.0 mmol) in THF (20 mL) was added over 0.5 hour. The reaction mixture was then allowed to warm to –10 °C over 14 hours, and then the reaction was quenched with NH_4Cl (sat., 10 mL). The mixture was extracted with

EtOAc (100 mL + 2 (50 mL), the combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 6:1 (4:1) to yield hydrazone **26** as a yellow oil (4.88 g, 84%). TLC R_f = 0.38 (silica, hexanes:EtOAc 5:1); $[\alpha]_D22$ –61 (*c* 1.45, $CHCl_3$); IR (film) ν_{max} 2926, 1454, 1097, 736, 697 cm^{-1}; MALDI-FTMS *m/z* 387.3008 (MH^+), calcd for $C_{24}H_{39}N_2O_2$ 387.3006.

Aldehyde 17. A solution of hydrazone **26** (3.82 g, 9.9 mmol) in iodomethane (10 mL) was heated at 60 °C (reflux condenser) for 3 hours, and was then cooled to 25 °C. Excess iodomethane was evaporated and traces removed under oil pump vacuum. The residual yellow syrup was vigorously stirred with 3 N HCl (190 mL) and pentane (190 mL) for 3 h at 25 °C, the phases were separated, and the aqueous phase was extracted with pentane (100 mL). The combined organic phase was dried (Na_2SO_4, $NaHCO_3$) and evaporated to yield aldehyde **17** as a yellow oil (2.38 g, 88%). $[\alpha]_D22$ +2 (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 2931, 2856, 1724, 1454, 1095, 1074, 736, 698 cm^{-1}; MALDI-FTMS *m/z* 297.1830 (MNa^+), calcd for $C_{18}H_{26}O_2Na$ 297.1825.

Due to the configurational lability at C8 (epothilone numbering), the aldehyde should be used immediately in the next step. The dr at C8 was estimated as follows: A sample of **17** was treated with excess $NaBH_4$ in methanol for 10 min. The reaction was quenched with NH_4Cl (sat.), the mixture was extracted with EtOAc, and the extract was dried (Na_2SO_4) and evaporated. The residue was treated with (*R*)-(–)-MTPACl (2-3 equiv.), excess triethylamine and 4-DMAP in CH_2Cl_2 for 3 hours. Purification by preparative TLC yielded a sample of the (*S*)-MTPA ester, which by ^1H NMR analysis showed a dr = 97:3, with the correct absolute stereochemistry at C8 as the major isomer (Tsuda, M., et al., *J. Org. Chem.* **2000**, *65*, 1349-1352). Analogous results were obtained by using (*S*)-(+)-MTPACl.

Aldol product 27. A solution of LDA was prepared by adding *n*-BuLi (7.5 mL, 12 mmol, 1.6 M in hexanes) to diisopropylamine (1.68 mL, 12 mmol) in THF (12 mL) at –78 °C, then warming the solution briefly to 0 °C, and finally cooling back to –78 °C. A solution of ketone **19** (4.63 g, 11.5 mmol) (Nicolaou, K. C., et al., *J. Am. Chem. Soc.* **1997**, *119*, 7974-7991) in THF (12 mL) was added dropwise over 2 min, and the

mixture was stirred for 1 h at –78 °C and then for 0.5 h at –40 °C. It was again cooled to –78 °C, and a solution of aldehyde **17** (1.37 g, 5.0 mmol) in THF (25 mL), pre-cooled to –78 °C, was added via cannula over 1 min, taking care to ensure minimal warming during transfer. The mixture was stirred for 5 min, and the reaction was then quenched by rapid injection of a solution of AcOH (1.4 mL) in THF (4.2 mL). After 5 min at –78 °C, the mixture was warmed to 25 °C and partitioned between NH_4Cl (sat., 50 mL) and ether (50 mL). The aqueous phase was extracted with ether (2 (50 mL), the combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:ether 20:1 (6:1) to yield recovered ketone **19** (1.71 g, 4.25 mmol) followed by the aldol product **27** in diastereomerically pure form (2.73 g, 81%). TLC R_f = 0.34 (silica, hexanes:EtOAc 5:1); $[\alpha]_D22$ –40 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3502 (br), 2954, 2928, 2856, 1681, 1472, 1255, 1098, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 699.4796 (MNa^+), calcd for $C_{39}H_{72}O_5Si_2Na$ 699.4816.

Alcohol 28. A solution of aldol product **27** (2.71 g, 4.0 mmol) and 2,6-lutidine (1.40 mL, 12 mmol) in CH_2Cl_2 (25 mL) was cooled to –20 °C and then TBSOTf (1.84 mL, 8.0 mmol) was added dropwise. The mixture was stirred for 1 h at –20 °C and the reaction was then quenched by the addition of NH_4Cl (sat., 25 mL). The mixture was warmed to 25 °C, the phases were separated and the aqueous phase was extracted with CH_2Cl_2 (25 mL) and ether (25 mL). The combined organic phase was dried (Na_2SO_4) and evaporated, and the residue was filtered through a plug of silica eluting with hexane:ether 10:1. The filtrate was evaporated and the resulting crude silyl ether (3.14 g, 4.0 mmol, 99%) was dissolved in THF (40 mL). To this was added a cold (0 °C) solution of HF·pyridine complex (6.4 mL) and pyridine (18 mL) in THF (32 mL) at 0 °C (this solution was prepared by slowly adding the HF·pyridine complex to a solution of pyridine in THF at 0 °C; ***CAUTION!*** *HF·pyridine is highly corrosive. The addition of HF·pyridine to the pyridine-THF solution is highly exothermic, and must be done with stirring and cooling in ice bath to prevent splashing*), and the resulting solution was stirred at 25 °C for 4 hours. The mixture was diluted with EtOAc (100 mL), placed in an ice bath, and quenched by the careful addition of $NaHCO_3$ (sat., 100 mL) and as much solid $NaHCO_3$ as needed to ensure complete neutralization

(***CAUTION!*** *Foaming!*). The mixture was extracted with EtOAc (3 (100 mL), and the combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 5:1) to yield **28** as a colorless oil (2.40 g, 89%). TLC R_f = 0.39 (silica, hexanes:EtOAc 5:1); $[\alpha]_D22$ –26 (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3458 (br), 2929, 2856, 1693, 1472, 1462, 1255, 1093, 986, 836, 775 cm^{-1}; MALDI-FTMS m/z 699.4807 (MNa^+), calcd for $C_{39}H_{72}O_5Si_2Na$ 699.4816.

Ester 29. The alcohol **28** (2.40 g, 3.5 mmol), Dess-Martin periodinane (3.75 g, 8.8 mmol), $NaHCO_3$ (0.74 g, 8.8 mmol) and water (76 µL, 4.2 mmol) were mixed in CH_2Cl_2 (80 mL), and the resulting suspension was stirred for 1 hour. The mixture was diluted with ether (200 mL), water (100 mL) and $NaHCO_3$ (sat., 100 mL), and was then filtered. The phases were separated and the aqueous phase was extracted with ether (2 (100 mL). The combined extract was dried (Na_2SO_4) and evaporated, and the residue was filtered through a plug of silica eluting with hexanes:EtOAc 6:1. The filtrate was evaporated and the resulting crude aldehyde (2.15 g, 3.2 mmol, 90%) was dissolved in a mixture of THF (80 mL), *t*-BuOH (145 mL) and 2-methyl-2-butene (25 mL). To this solution was added a solution of NaH_2PO_4 (0.95 g, 6.7 mmol) and $NaClO_2$ (1.14 g, 10 mmol) in water (31 mL), and the resulting mixture was stirred vigorously for 1 hour. The volatiles were removed by evaporation, and the residue was partitioned between EtOAc (100 mL) and brine (100 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 (100 mL). The combined extract was dried (Na_2SO_4) and evaporated, and the residue was dissolved in DMF (5 mL) and evaporated again to remove traces of *t*-BuOH. The so obtained crude acid (2.4 g, *ca.* 3.2 mmol >100%,) was again dissolved in DMF (10 mL), to which 2-(trimethylsilyl)ethanol (1.83 mL, 12.7 mmol), EDC (0.92 g, 4.8 mmol), and 4-DMAP (40 mg, 0.33 mmol) were added. The resulting suspension was stirred for 14 hours, after which time a clear solution was obtained. Water (10 mL) was added and the mixture was extracted with ether (3 (50 mL). The combined extract was washed with water-brine mixture (100 + 100 mL), dried (Na_2SO_4) and evaporated. The residue was purified by flash chromatography (silica, hexanes:EtOAc 10:1) to yield ester **29** as a viscous, pale yellow oil (2.08 g, 74%). TLC R_f = 0.57 (silica, hexanes:EtOAc 10:1); $[\alpha]_D22$ –33 (*c* 1.2, $CHCl_3$); IR (film) ν_{max}

2954, 2930, 2856, 1735, 1695, 1472, 1385, 1252, 1090, 988, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 813.5315 (MNa^+), calcd for $C_{44}H_{82}O_6Si_3Na$ 813.5311.

Aldehyde 30. To a solution of benzyl ether **29** (2.08 g, 2.63 mmol) in EtOH:EtOAc 1:1 (50 mL) was added 20% $Pd(OH)_2$ on carbon (2.1 g, 60% moisture), and the mixture was hydrogenated for 1 hour. It was then filtered through celite to remove the catalyst, the filtrate was evaporated, and the residue was co-evaporated with benzene to remove traces of EtOH. The resulting crude alcohol (1.89 g, *ca.* 2.6 mmol, >100%) was dissolved in CH_2Cl_2 (60 mL), Dess-Martin periodinane (2.76 g, 6.5 mmol), $NaHCO_3$ (0.55 g, 6.5 mmol) and water (56 µL, 3.1 mmol) were added, and the resulting suspension was stirred for 1 hour. The mixture was diluted with ether (150 mL), water (75 mL) and $NaHCO_3$ (sat., 75 mL), and was then filtered. The phases were separated and the aqueous phase was extracted with ether (2 (75 mL). The combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 15:1) to yield aldehyde **30** as a viscous oil (1.55 g, 84%). TLC R_f = 0.24 (silica, hexanes:EtOAc 15:1); $[\alpha]_D22$ – 47 (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 2954, 2856, 1734, 1703, 1251, 1173, 1084, 988, 837, 776 cm^{-1}; MALDI-FTMS *m/z* 721.4671 (MNa^+), calcd for $C_{37}H_{74}O_6Si_3Na$ 721.4685.

Enol ether 31. To a suspension of $MeOCH_2PPh_3Cl$ (3.09 g, 9.0 mmol) in THF (20 mL) at 0 °C was added NaHMDS (8.5 mL, 8.5 mmol, 1 M in THF) dropwise. A red color developed. The mixture was stirred at 0 °C for 0.5 h and it was then cooled to –40 °C. A solution of aldehyde **30** (2.12 g, 3.0 mmol) in THF (7 mL) was added, and the mixture was allowed to warm to –10 °C over 2 hours. The reaction was quenched with NH_4Cl (sat., 15 mL), the phases were separated, and the aqueous phase was extracted with EtOAc (2 (75 mL). The combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 30:1) to yield enol ether **31** as a colorless, viscous oil (1.85 g, 84%, olefin *cis:trans ca.* 1:1 by ^1H NMR). TLC R_f = 0.23 (silica, hexanes:EtOAc 30:1); $[\alpha]_D22$ –36 (*c* 1.2, $CHCl_3$); IR (film) ν_{max} 2954, 2930, 2856, 1735, 1695, 1251, 1171, 1105, 988, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 749.4996 (MNa^+), calcd for $C_{39}H_{78}O_6Si_3Na$ 749.4998.

Aldehyde 32. To a solution of enol ether **31** (847 mg, 1.16 mmol) in dioxane:water 9:1 (12 mL) was added pyridinium *para*-toluenesulfonate (2.34 g, 9.31 mmol) and the mixture was stirred at 70 °C until TLC indicated the completion of the reaction (6-10 h). The reaction was then quenched with $NaHCO_3$ (sat., 15 mL), and the mixture was extracted with EtOAc (3 (50 mL). The combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 15:1) to yield **32** as a colorless, viscous oil (681 mg, 82%). TLC R_f = 0.29 (silica, hexanes:EtOAc 15:1); $[\alpha]_D$22 –34 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 2954, 2856, 1731, 1695, 1251, 1086, 988, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 735.4823 (MNa^+), calcd for $C_{38}H_{76}O_6Si_3Na$ 735.4842.

Construction of vinyl iodides 20c–g

2-Bromo-5-[(trityloxy)methyl]pyridine 34. Trityl chloride (3.90 g, 14 mmol), 4-DMAP (2.08 g, 17 mmol) and 2-bromo-5-hydroxymethylpyridine **33** (1.88 g, 10 mmol) (Ellingboe, J. W., et al., *J. Med. Chem.* **1994**, *37*, 542-550) were dissolved in DMF (15 mL) and the solution was stirred at 80 °C for 48 hours. A white precipitate formed during this time. After cooling, the mixture was diluted with $NaHCO_3$ (sat., 25 mL) and extracted with EtOAc (3 (50 mL). The combined extract was washed with brine, with a few drops of NaOH (1 M) added (2 (100 mL). After drying and evaporation, the solid residue was purified by flash chromatography (silica, hexanes:EtOAc 15:1) to yield **34** as a white solid (4.46 g, 100%). TLC R_f = 0.30 (silica, hexanes:EtOAc 15:1); IR (film) ν_{max} 3057, 1448, 1086, 764, 700, 632 cm^{-1}; MALDI-FTMS *m/z* 430.0792 (MH^+), calcd for $C_{25}H_{21}BrNO$ 430.0801.

Sonogashira coupling of aryl bromdes (34, 37, 38, and 39) with propyne (general procedure). To a briefly deoxygenated (Ar bubbling) solution of the aryl bromide **34**, **37**, **38**, or **39** (3.5 mmol) in DMF (3 mL) and diisopropyl amine (2.5 mL) were added $Pd(PPh_3)_2Cl_2$ (25 mg, 36 µmol) and CuI (13 mg, 70 µmol) under Ar(g), and then the inert atmosphere was replaced by propyne (1 atm, balloon). The mixture was stirred at 25 °C for 3 hours. During this time, a precipitate formed, and the reaction mixture turned dark brown. Water (15 mL) was added, the mixture was extracted with EtOAc, and the combined extract was dried (Na_2SO_4) and evaporated.

The pure 1-arylpropyne was obtained by flash chromatography (silica, hexane:EtOAc mixtures).

Propynylpyridine 35. Brown foam (96%); TLC R_f = 0.23 (silica, hexanes:EtOAc 5:1); IR (film) ν_{max} 3057, 2229, 1594, 1560, 1478, 1448, 1075, 702 cm^{-1}; MALDI-FTMS *m/z* 390.1851 (MH^+), calcd for $C_{28}H_{24}NO$ 390.1852.

Pyridine 36. A solution of trityl ether **35** (1.38 g, 3.54 mmol) in $CHCl_3$ (15 mL) was cooled to 0 °C and then saturated with HCl (g). After 1 h at 0 °C, the reaction was quenched by the addition of $NaHCO_3$ (sat., 50 mL), and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phase was dried (Na_2SO_4) and evaporated. Flash chromatography (silica, hexanes:EtOAc 1:2 + 5% MeOH) afforded **5-hydroxymethyl-2-prop-1-ynylpyridine** as a yellow, viscous oil (0.36 g, 69%). TLC R_f = 0.29 (silica, hexanes:EtOAc 1:2 + 5% MeOH); IR (film) ν_{max} 3262, 2916, 2230, 1596, 1561, 1023, 838 cm^{-1}; MALDI-FTMS *m/z* 148.0754 (MH^+), calcd for $C_9H_{10}NO$ 148.0757. To a solution of this alcohol (0.40 g, 2.7 mmol) in THF (10 mL) at 0 °C was added NaH (0.13 g, 3.3 mmol, 60% in oil). After stirring for 5 min, chloromethyl methyl ether (0.25 mL, 3.3 mmol) was added, and the mixture was stirred at 0 °C for 1 hour. The reaction was then quenched with NaCl (sat.), and a few drops of NaOH (1 M) were added. The mixture was extracted with EtOAc (3 (50 mL), the combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc 1:1) to yield **36** as a pale yellow oil (0.26 g, 50%). TLC R_f = 0.41 (silica, hexanes:EtOAc 1:1); IR (film) ν_{max} 2947, 2230, 1595, 1560, 1478, 1149, 1104, 1047, 919, 830 cm^{-1}; MALDI-FTMS *m/z* 192.1014 (MH^+), calcd for $C_{11}H_{14}NO_2$ 192.1019.

Sonogashira coupling product from 37. The reaction was very slow, probably due to Pd coordination to the thioether moiety; therefore, 10 mol% $Pd(PPh_3)_2Cl_2$ and 20 mol% CuI were used. The product was obtained as a brown oil (42%). TLC R_f = 0.37 (silica, hexanes:EtOAc 15:1); IR (film) ν_{max} 3110, 2914, 2240, 1493, 1417, 1278, 1037, 966, 735 cm^{-1}; MALDI-FTMS *m/z* 170.0092 (MH^+), calcd for $C_7H_8NS_2$ 170.0093.

Sonogashira coupling product from 38. Brown oil (97%); TLC R_f = 0.21 (silica, hexanes:EtOAc 5:1); IR (film) ν_{max} 2908, 2226, 1567, 1531, 1461, 1431, 1361, 1108, 1008, 832 cm^{-1}; MALDI-FTMS *m/z* 164.0527 (MH^+), calcd for $C_9H_{10}NS$ 164.0528.

Sonogashira coupling product from 39. Yellow oil (70%); TLC R_f = 0.36 (silica, hexanes:EtOAc 20:1); IR (film) ν_{max} 2924, 2231, 1566, 1554, 1431, 1156, 1140, 790 cm^{-1}; MALDI-FTMS *m/z* 164.0526 (MH^+), calcd for $C_9H_{10}NS$ 164.0528.

Hydrostannylation-iodination (general procedure). This is an adaption of the previously reported procedure (Betzer, J.-F., et al., *Tetrahedron Lett.* **1997**, *38*, 2279-2282). To a solution of hexabutylditin (10.1 mL, 20 mmol) in dry THF (40 mL) at –78 °C was added *n*-BuLi (12.9 mL, 20 mmol, 1.55 M in hexanes), and the resulting clear solution was stirred at –40 °C for 30 min. It was then transferred via cannula to a suspension of CuCN (0.90 g, 10 mmol) in THF (2 mL) at –78 °C. A clear yellow solution formed, and it was stirred for 5 min at –40 °C before being re-cooled to –78 °C. Then dry methanol (23 mL, 0.57 mol) was added to yield a red solution, which was stirred at –40 °C for 15 min, after which a solution of the arylpropyne (5.0 mmol) in THF (5 mL) was added. The orange-red solution was stirred at –10 °C overnight (some Cu and/or Cu^{2+} salts precipitate), then cooled to –20 °C, followed by the addition of methanol (10 mL). After 15 min at –20 °C, water (10 mL) was added, and stirring was continued for another 15 min, while warming to 25 °C. The mixture was extracted with ether, and the organic phase was washed with brine, dried (Na_2SO_4) and evaporated. Flash chromatography (silica, hexanes:EtOAc mixtures) yielded the intermediate vinylstannane, which was dissolved in CH_2Cl_2 (5 mL). A solution of iodine (1.05 equiv.) in CH_2Cl_2 (40 mL per g I_2) was then added dropwise to this solution at 0 °C. After the last few drops, the color of I_2 persisted, and the reaction was allowed to continue for another 5 min at 0 °C. Then the solvent was evaporated and the residue was dissolved in ether. KF (1 M solution in water, 3 equiv.) and $Na_2S_2O_3$ (sat., 10 mL per mmol substrate) were added, and the mixture was stirred for 15 min at 25 °C during which time a white precipitate formed. The mixture was filtered through celite, and the organic phase was dried (Na_2SO_4) and evaporated.

The residue was purified by flash chromatography (silica, hexanes:EtOAc mixtures) to yield the desired vinyl iodide.

Vinyl iodide 20c. White cloudy film (80%). TLC R_f = 0.25 (silica, hexanes:EtOAc 20:1); IR (film) ν_{max} 3060, 2919, 1619, 1596, 1484, 1443, 1373, 1214, 1061, 985, 873, 761, 703, 632 cm^{-1}; MALDI-FTMS *m/z* 518.0990 (MH^+), calcd for $C_{28}H_{25}INO$ 518.0975.

Vinyl iodide 20d. Yellow oil (67%). TLC R_f = 0.51 (silica, hexanes:EtOAc 4:1); IR (film) ν_{max} 2924, 1716, 1619, 1596, 1481, 1372, 1211, 1149, 1102, 1045, 918, 873, 609, 517 cm^{-1}; MALDI-FTMS *m/z* 320.0142 (MH^+), calcd for $C_{11}H_{15}INO_2$ 320.0142.

Vinyl iodide 20e. The intermediate vinyl stannane is readily protodestannylated; therefore, flash chromatography of this intermediate must be performed using hexanes:EtOAc:Et_3N 50:1:1 as eluent, and the so obtained vinylstannane contained other butyl tin compounds. Following the general procedure, the mixture was treated with enough I_2 that the brown color persisted at the end of the addition (*ca.* 2 equiv. of I_2). After flash chromatography (hexanes:EtOAc 50:1), vinyl iodide **20e** was obtained as a yellow oil (74%). TLC R_f = 0.41 (silica, hexanes:EtOAc 50:1); IR (film) ν_{max} 3102, 2923, 1620, 1423, 1300, 1065, 1035, 964, 863, 723, 562 cm^{-1}; MALDI-FTMS *m/z* 297.9215 (MH^+), calcd for $C_7H_9INS_2$ 297.9216.

Vinyl iodide 20f. Yellow solid (80%). TLC R_f = 0.19 (silica, hexanes:EtOAc 40:1); IR (film) ν_{max} 2919, 1619, 1567, 1467, 1431, 1373, 1108, 1067, 1014, 961, 867, 820, 521 cm^{-1}; MALDI-FTMS *m/z* 291.9655 (MH^+), calcd for $C_9H_{11}INS$ 291.9651.

Vinyl iodide 20g. Yellow oil (83%). TLC R_f = 0.28 (silica, hexanes:EtOAc 40:1); IR (film) ν_{max} 2919, 1620, 1549, 1425, 1155, 1138, 1061, 991, 961, 861, 785, 732, 550 cm^{-1}; MALDI-FTMS *m/z* 291.9653 (MH^+), calcd for $C_9H_{11}INS$ 291.9651.

Synthesis of epothilone analogues 8–144.

Nozaki-Hiyama-Kishi coupling of aldehydes (34, 40) with vinyl stannanes (20a–g) (general procedure). To a briefly vacuum-degassed solution of aldehyde **32** (107 mg, 0.15 mmol), the requisite vinyl iodide **20** (0.45 mmol), and 4-*tert*-butylpyridine

(665 µL, 4.5 mmol) in DMSO (3 mL) were added anhydrous $CrCl_2$ (184 mg, 1.5 mmol) and anhydrous $NiCl_2$ (4 mg, 0.03 mmol). The mixture was stirred at 25 °C for 3 hours, after which another portion of vinyl iodide (0.45 mmol) was added, and stirring was continued for a further 3 hours. This was repeated one more time, after which stirring was continued overnight. The reaction was then quenched with water (5 mL), pyridine (1 mL) was added to prevent Cr-product complexes from being extracted into the water phase, and the mixture was extracted with EtOAc (3 (25 mL). The combined extract was washed with brine (2 (100 mL), dried (Na_2SO_4) and evaporated. Flash chromatography (silica, hexanes:EtOAc mixtures) yielded the coupling product, in most cases inseparable from excess 4-*tert*-butylpyridin.

Product from 20a and 32. Yellow oil (85% as a *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.26 (silica, hexanes:EtOAc 4:1); $[\alpha]_D22$ –25 (*c* 0.36, CH_2Cl_2); IR (film) ν_{max} 2943, 2860, 1731, 1696, 1467, 1384, 1290, 1249, 1173, 1079, 985, 832, 773 cm^{-1}; MALDI-FTMS *m/z* 860.5128 (MNa^+), calcd for $C_{44}H_{83}NO_6SSi_3Na$ 860.5141.

Product from 20b and 32. This coupling product was inseparable from 4-*tert*-butyl pyridine, and was subjected to the TBAF deprotection conditions (vide infra) as a crude mixture.

Product from 20d and 32. This coupling product was inseparable from 4-*tert*-butyl pyridine, and was subjected to the TBAF deprotection conditions (vide infra) as a crude mixture.

Product from 20e and 32. Yellow glass (78%, *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.40 (silica, hexanes:EtOAc 5:1); $[\alpha]_D22$ –28 (*c* 2.0, $CHCl_3$); IR (film) ν_{max} 3416 (br), 2929, 2856, 1732, 1694, 1472, 1251, 1037, 988, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 906.5021 (MH^+), calcd for $C_{45}H_{85}NO_6S_2Si_3Na$ 906.5018.

Product from 20f and 32. This coupling product was inseparable from 4-*tert*-butyl pyridine, and was subjected to the TBAF deprotection conditions (vide infra) as a crude mixture.

Product from 20g and 32. This coupling product was inseparable from 4-*tert*-butyl pyridine, and was subjected to the TBAF deprotection conditions (vide infra) as a crude mixture.

Product from 20c and 40. Yellow glass (87% for two steps from aldehyde **40** as a *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.15 (silica, hexanes:EtOAc 4:1); $[\alpha]_D$22 -23 (*c* 0.19, CH_2Cl_2); IR (film) ν_{max} 2931, 2861, 1731, 1690, 1467, 1384, 1355, 1249, 1167, 1061, 985, 832, 773, 703 cm^{-1}; MALDI-FTMS *m/z* 1112.6634 (MNa^+), calcd for $C_{65}H_{99}NO_7Si_3Na$ 1112.6621.

Product from 20e and 40. Colorless glass (59%, *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.27 (silica, hexanes:EtOAc 5:1); $[\alpha]_D$22 –28 (*c* 2.0, $CHCl_3$); IR (film) ν_{max} 3396 (br), 2928, 2855, 1734, 1693, 1472, 1251, 1037, 988, 836, 775 cm^{-1}; MALDI-FTMS *m/z* 892.4861 (MNa^+), calcd for $C_{44}H_{83}NO_6S_2Si_3Na$ 892.4862.

TBAF deprotection (general procedure). The product mixture from the Nozaki-Hiyama-Kishi coupling was dissolved in THF (1.5 mL), and TBAF (1 M in THF, 0.30 mL, 0.30 mmol) was added at 0 °C. After 1 h at 0 °C, another portion of TBAF (0.30 mL, 0.30 mmol) was added, and the mixture was stirred at 25 °C for 1 hour. The reaction was quenched with NH_4Cl (sat., 5 mL), and the mixture was extracted with EtOAc (4 (20 mL). The combined extract was dried (Na_2SO_4) and evaporated, and the residue was purified by flash chromatography (silica, hexanes:EtOAc mixtures) to yield the desired hydroxy acid as a *ca.* 1:1 mixture of C15 epimers (inseparable at this stage).

Hydroxy acid 41a. The reaction mixture from the deprotection was quickly filtered through a plug of silica gel, and this crude product (73% yield from aldehyde **32**) was subjected to the Yamaguchi macrolactonization (*vide infra*) without further purification.

Hydroxy acid 41b. Yellow solid (57%, *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.19 (silica, hexanes:EtOAc 2:1); $[\alpha]_D$22 –6 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3369 (br), 2930, 2857, 1783, 1694, 1471, 1251, 1085, 1084, 988, 836, 775 cm^{-1}; MALDI-FTMS *m/z* 768.5028 (MNa^+), calcd for $C_{42}H_{75}NO_6Si_2Na$ 768.5025.

Hydroxy acid 41d. Yellow glass (49% for 2 steps from aldehyde **32** as a *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.20 (silica, hexanes:EtOAc 1:1); $[\alpha]_D$22 +1 (*c* 0.19, CH_2Cl_2); IR (film) ν_{max} 2933, 2858, 1694, 1600, 1563, 1463, 1382, 1357, 1251, 1145, 1096, 1046, 989, 834, 772, 666 cm^{-1}; MALDI-FTMS *m/z* 806.5437 (MH^+), calcd for $C_{44}H_{80}NO_8Si_2$ 806.5417.

Hydroxy acid 41e. Yellow solid (79%, *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.37 (silica, hexanes:EtOAc 2:1); $[\alpha]_D$22 –23 (*c* 2.3, $CHCl_3$); IR (film) ν_{max} 3356 (br), 2929, 2856, 1712, 1472, 1253, 1085, 1038, 988, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 806.4282 (MNa^+), calcd for $C_{40}H_{73}NO_6S_2Si_2Na$ 806.4315.

Hydroxy acid 41f. Colorless glass (63%, *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.21 (silica, hexanes:EtOAc 2:1); $[\alpha]_D$22 –3 (*c* 0.44, CH_2Cl_2); IR (film) ν_{max} 2933, 2858, 1693, 1467, 1253, 1086, 984, 833, 774 cm^{-1}; MALDI-FTMS *m/z* 800.4754 (MNa^+), calcd for $C_{42}H_{75}NO_6SSi_2Na$ 800.4746.

Hydroxy acid 41g. Yellow glass (46% for 2 steps from aldehyde **32** as a *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.46 (silica, hexanes:EtOAc 2:1); $[\alpha]_D$22 –11 (*c* 0.19, CH_2Cl_2); IR (film) ν_{max} 2933, 2858, 1706, 1557, 1463, 1426, 1364, 1251, 1083, 989, 834, 772, 666 cm^{-1}; MALDI-FTMS *m/z* 800.4746 (MNa^+), calcd for $C_{42}H_{75}NO_6SSi_2Na$ 800.4746.

Hydroxy acid 42c. The reaction mixture from the deprotection was quickly filtered through a plug of silica gel, and this crude product (46% yield from aldehyde **40**) was subjected to the Yamaguchi macrolactonization (vide infra) without further purification.

Hydroxy acid 42e. Pale yellow glass (66%, *ca.* 1:1 mixture of C15 epimers). TLC R_f = 0.39 (silica, hexanes:EtOAc 2:1); $[\alpha]_D$22 –20 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3354 (br), 2928, 2856, 1713, 1471, 1253, 1087, 988, 836, 775 cm^{-1}; MALDI-FTMS *m/z* 792.4161 (MNa^+), calcd for $C_{39}H_{71}NO_6S_2Si_2Na$ 792.4153.

Yamaguchi macrolactonization (general procedure). To a solution of the hydroxy acid (95 µmol) in dry THF (8 ml) at 0 °C was added triethylamine (79 µl, 0.57 mmol)

and 2,4,6-trichlorobenzoyl chloride (40 µl, 0.23 mmol). After stirring at 0 °C for 1 hour, the resulting solution was added over 2 h to a solution of 4-DMAP (26 mg, 0.21 mmol) in toluene (20 mL) at 75 °C using a syringe pump. Stirring was continued at 75 °C for another 1 h after which the toluene was evaporated under reduced pressure. The residue was directly subjected to flash chromatography (silica, hexanes:EtOAc mixtures) to yield the macrolactone and its (15*R*)-epimer, readily separable. In all cases the desired (15*S*)-epimer eluted *after* the less polar (15*R*)-epimer.

Macrolactone 43a. colorless glass (28% for two steps from the Nozaki-Hiyama-Kishi coupling product of aldehyde **32** and vinyl iodide **20a.**); TLC R_f = 0.21 (silica, hexanes:EtOAc 20:1); $[\alpha]_D$22 –33 (*c* 0.56, CH_2Cl_2); IR (film) ν_{max} 2932, 2855, 1739, 1689, 1465, 1383, 1252, 1181, 1153, 1099, 1066, 1017, 984, 869, 836, 776 cm^{-1}; MALDI-FTMS *m/z* 734.4639 (MH^+), calcd for $C_{40}H_{72}NO_5SSi_2$ 734.4664.

Macrolactone 43b. Colorless glass (28%); TLC R_f = 0.27 (silica, hexanes:EtOAc 10:1); $[\alpha]_D$22 –28 (c 1.0, $CHCl_3$); IR (film) ν_{max} 2929, 2856, 1740, 1695, 1472, 1384, 1253, 1100, 1020, 986, 836, 775 cm^{-1}; MALDI-FTMS *m/z* 728.5109 (MH^+), calcd for $C_{42}H_{74}NO_5Si_2$ 728.5106.

Macrolactone 43d. Yellow glass (35%); TLC R_f = 0.14 (silica, hexanes: EtOAc 6:1); $[\alpha]_D$22 –28 (*c* 0.12, CH_2Cl_2); IR (film) ν_{max} 2931, 2861, 1737, 1690, 1596, 1467, 1378, 1249, 1149, 1102, 1049, 985, 832, 773 cm^{-1}; MALDI-FTMS *m/z* 810.5116 (MNa^+), calcd for $C_{44}H_{77}NO_7Si_2Na$ 810.5130.

Macrolactone 43e. This product was isolated as a crude mixture which was directly subjected to the global desilylation conditions (*vide infra*) without further purification.

Macrolactone 43f. Colorless glass (45%); TLC R_f = 0.20 (silica, hexanes:EtOAc 10:1); $[\alpha]_D$22 -0.30 (*c* 0.10, CH_2Cl_2); IR (film) ν_{max} 2933, 285, 1737, 1668, 1463, 1382, 1357, 1251, 1102, 1015, 983, 871, 834, 772 cm^{-1}; MALDI-FTMS *m/z* 760.4799 (MH^+), calcd for $C_{42}H_{74}NO_5SSi_2$ 760.4820.

Macrolactone 43g Yellow glass (37%); TLC R_f = 0.47 (silica, hexanes:EtOAc 10:1); $[\alpha]_D22$ –14 (*c* 0.31, $CHCl_3$); IR (film) ν_{max} 2929, 2856, 1740, 1696, 1557, 1461, 1431, 1379, 1250, 1099, 107, 979, 836, 774 cm^{-1}; MALDI-FTMS *m/z* 760.4802 (MH^+), calcd for $C_{42}H_{74}NO_5SSi_2$ 760.4820.

Macrolactone 44c. Colorless glass (33% for 2 steps from the Nozaki-Hiyama-Kishi coupling product of aldehyde **40** and vinyl iodide **20c**); TLC R_f = 0.46 (silica, hexanes:EtOAc 10:1); $[\alpha]_D22$ –17 (*c* 0.56, CH_2Cl_2); IR (film) ν_{max} 2931, 2861, 1743, 1696, 1467, 1378, 1249, 1161, 1073, 1020, 985, 873, 833, 773, 703, 579 cm^{-1}; MALDI-FTMS *m/z* 972.5969 (MH^+), calcd for $C_{60}H_{86}NO_6Si_2$ 972.5988.

Macrolactone 44e. Colorless glass (47%); TLC R_f = 0.31 (silica, hexanes:EtOAc 15:1); $[\alpha]_D22$ –19 (*c* 0.50, $CHCl_3$); IR (film) ν_{max} 2929, 2855, 1741, 1697, 1472, 1254, 1102, 1036, 986, 836, 775 cm^{-1}; MALDI-FTMS *m/z* 774.4056 (MNa^+), calcd for $C_{39}H_{69}NO_5S_2Si_2Na$ 774.4048.

Global desilylation (general procedure). The macrolactone was dissolved in 20% v/v TFA in CH_2Cl_2, and the solution was kept at 25 °C for 3 hours, after which the volatiles were evaporated without heating. The residue was dissolved in EtOAc, and the solution was washed with $NaHCO_3$ (sat.), dried (Na_2SO_4) and evaporated. Flash chromatography (silica, hexanes:EtOAc mixtures) afforded the pure epothilone.

Epothilone 6. Colorless glass (73%); TLC R_f = 0.25 (silica, hexanes:EtOAc 2:1); $[\alpha]_D22$ –34 (*c* 0.11, CH_2Cl_2); IR (film) ν_{max} 3472 (br), 2931, 1732, 1684, 1456, 1378, 1258, 1179, 1149, 1067, 1043, 1012, 973, 873, 732 cm^{-1}; MALDI-FTMS *m/z* 506.2931 (MH^+), calcd for $C_{28}H_{44}NO_5S$ 506.2935.

Epothilone 8. Colorless glass (48%); TLC R_f = 0.52 (silica, hexanes:EtOAc 1:1); $[\alpha]_D22$ –54 (*c* 0.30, $CHCl_3$); IR (film) ν_{max} 3445 (br), 2936, 1732, 1682, 1454, 1383, 1259, 756 cm^{-1}; MALDI-FTMS *m/z* 500.3369 (MH^+), calcd for $C_{30}H_{46}NO_5$ 500.3376.

Epothilone 10. The general procedure failed to cleave the MOM protecting group cleanly. Therefore, this group was first removed using bromotrimethylsilane as follows: To a solution of protected epothilone **43d** (11 mg, 14 µmol) in dry CH_2Cl_2

(0.4 mL) was added powdered 4Å MS (5 mg), and the resulting mixture was cooled to –30 °C. Bromotrimethylsilane (18.4 µL, 140 µmol) was added dropwise, and the mixture was stirred at –30 °C for 1 hour, after which the reaction was quenched with $NaHCO_3$ (sat.) and extracted five times with EtOAc. The combined extract was dried and evaporated, and the residue subjected to the general desilylation procedure to yield **10** as a colorless glass (56%); TLC R_f = 0.42 (silica, hexanes:EtOAc 1:4); $[\alpha]_D22$ –52 (*c* 0.12, CH_2Cl_2); IR (film) ν_{max} 3401 (br), 2931, 1731, 1684, 1596, 1561, 1461, 1378, 1331, 1290, 1255, 1173, 1149, 1044, 1008, 979, 879, 732 cm^{-1}; MALDI-FTMS *m/z* 516.3330 (MH^+), calcd for $C_{30}H_{46}NO_6$ 516.3319.

Epothilone 12. Viscous oil (17% from **41e**); TLC R_f = 0.38 (silica, hexanes:EtOAc 2:1); $[\alpha]_D22$ –52 (*c* 0.50, $CHCl_3$); IR (film) ν_{max} 3490 (br), 2933, 1732, 1686, 1255, 1038, 756 cm^{-1}; MALDI-FTMS *m/z* 538.2666 (MH^+), calcd for $C_{28}H_{44}NO_5S_2$ 538.2655.

Epothilone 13. Colorless glass (68%); TLC R_f = 0.57 (silica, hexanes:EtOAc 1:1); $[\alpha]_D22$ –46 (*c* 0.34, CH_2Cl_2); IR (film) ν_{max} 3484 (br), 2932, 1731, 1684, 1469, 1367, 1255, 1150, 1044, 1009, 973, 879, 826, 732 cm^{-1}; MALDI-FTMS *m/z* 554.2915 (MNa^+), calcd for $C_{30}H_{45}NO_5SNa$ 554.2910.

Epothilone 14. Colorless glass (48%); TLC R_f = 0.42 (silica, hexanes:EtOAc 2:1); $[\alpha]_D22$ –38 (*c* 0.34, CH_2Cl_2); IR (film) ν_{max} 3478 (br), 2930, 1732, 1682, 1556, 1434, 1378, 1257, 1149, 1137, 1067, 1044, 1012, 979, 785, 732 cm^{-1}; MALDI-FTMS *m/z* 532.3078 (MH^+), calcd for $C_{30}H_{45}NO_5S$ 532.3091.

Epothilone 9. Colorless glass (54%); TLC R_f = 0.13 (silica, hexanes:EtOAc 1:2); $[\alpha]_D22$ -24 (*c* 0.14, CH_2Cl_2); IR (film) ν_{max} 3379, 2920, 2857, 1725, 1688, 1600, 1459, 1370, 1255, 1151, 1047, 1010, 979, 880, 734 cm^{-1}; MALDI-FTMS *m/z* 524.3004 (MNa^+), calcd for $C_{29}H_{43}NO_6Na$ 524.2982.

Epothilone 11. Colorless glass (68%); TLC R_f = 0.28 (silica, hexanes:EtOAc 2:1); $[\alpha]_D22$ –26 (*c* 0.30, $CHCl_3$); IR (film) ν_{max} 3444 (br), 2925, 1731, 1693, 1454, 1258, 1037, 756 cm^{-1}; MALDI-FTMS *m/z* 546.2330 (MNa^+), calcd for $C_{27}H_{41}NO_5S_2Na$ 546.2318.

Compound 48: R_f = 0.19 (silica gel, ethyl acetate/hexanes = 3/7); $[\alpha]_D^{20}$ -19.3 (c 0.14, CH_2Cl_2); IR (film): ν_{max} 3484 (br), 2932, 1729, 1459, 1375, 1249, 1043, 982, 733 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.97 (s, 1 H), 6.47 (s, 1 H), 5.25 (dd, *J* = 5.7, 7.1 Hz, 1 H), 4.04 (dd, *J* = 3.0, 8.1 Hz, 1 H), 3.91 (dd, *J* = 4.1, 4.1 Hz, 1 H), 3.23 (m, 1 H), 2.69 (s, 3 H), 2.52 (dd, *J* = 8.4, 14.9 Hz, 1 H), 2.46 (dd, *J* = 2.6, 14.9 Hz, 1 H), 2.11 (s, 3 H), 2.04 (dd, *J* = 4.0, 14.5 Hz, 1 H), 1.66-1.72 (m, 1 H), 1.44-1.62 (m, 4 H), 1.36 (s, 3 H), 1.22-1.35 (m, 2 H), 1.17 (d, *J* = 7.5 Hz, 3 H), 1.16 (s, 3 H), 1.04-1.15 (m, 1 H), 0.99 (d, *J* = 7.0 Hz, 3 H), 0.97 (s, 3 H), 0.48 (m, 1 H), 0.40 (dd, *J* = 3.9, 8.8 Hz, 1 H), -0.11 ppm (br t, *J* = 4.6 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 221.5, 171.1, 165.7, 152.9, 138.6, 120.1, 116.2, 82.0, 73.8, 73.2, 52.0, 42.9, 39.4, 36.5, 35.0, 33.2, 31.6, 24.6, 23.5, 22.54, 22.49, 21.1, 20.8, 19.4, 17.4, 16.8, 15.0, 13.2 ppm; MALDI-FTMS: m/z 538.2632 (MH^+), calcd for $C_{28}H_{44}NO_5S_2$ 538.2655.

Compound 50: R_f = 0.27 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -61 (c 0.12, CH_2Cl_2); MALDI-FTMS: m/z 500.3376 (MH^+), calcd for $C_{30}H_{46}NO_5$ 500.3370.

Compound 51: R_f = 0.37 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -44 (c 0.14, CH_2Cl_2); MALDI-FTMS: m/z 554.2604 (MH^+), calcd for $C_{28}H_{44}NO_6S_2$ 554.2604.

Compound 52: R_f = 0.31 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -32 (c 0.33, CH_2Cl_2); MALDI-FTMS: m/z 608.2334 (MH^+), calcd for $C_{28}H_{41}F_3NO_6S_2$ 608.2322.

Compound 53: R_f = 0.38 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -43 (c 0.12, CH_2Cl_2); MALDI-FTMS: m/z 568.2777 (MH^+), calcd for $C_{29}H_{46}NO_6S_2$ 568.2761.

Compound 54: R_f = 0.27 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -28 (c 0.26, CH_2Cl_2); MALDI-FTMS: m/z 628.2376 (MNa^+), calcd for $C_{31}H_{43}NO_7S_2Na$ 628.2373.

Compound 55: R_f = 0.24 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -49 (c 0.45, CH_2Cl_2); MALDI-FTMS: m/z 566.2116 (MH^+), calcd for $C_{28}H_{41}BrNO_6$ 566.2112.

Compound 56: R_f = 0.36 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -27 (c 0.15, CH_2Cl_2); MALDI-FTMS: m/z 544.2419 (MNa^+), calcd for $C_{28}H_{40}ClNO_6Na$ 544.2436.

Compound 57: R_f = 0.28 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -49 (c 0.45, CH_2Cl_2); MALDI-FTMS: m/z 534.2907 (MH^+), calcd for $C_{29}H_{44}NO_6S$ 534.2884.

Compound 58: R_f = 0.35 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -50 (c 0.62, CH_2Cl_2); MALDI-FTMS: m/z 556.2724 (MNa^+), calcd for $C_{29}H_{43}NO_6SNa$ 556.2703.

Compound 59: R_f = 0.37 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -34 (c 0.24, CH_2Cl_2); MALDI-FTMS: m/z 556.2891 (MH^+), calcd for $C_{29}H_{41}F_3NO_6$ 556.2880.

Compound 60: R_f = 0.34 (silica gel, ethyl acetate/hexanes = 1/1); $[\alpha]_D^{20}$ -33 (c 0.80, CH_2Cl_2); MALDI-FTMS: m/z 535.2820 (MH^+), calcd for $C_{28}H_{43}N_2O_6S$ 535.2836.

Example : Soft capsules

5000 soft gelatin capsules, each containing as active ingredient 0.05 g of one of the compounds of formula I named in the preceding examples, e.g. the compounds of example 1, 2, 3 or 4, are prepared as follows:

composition	
active ingredient	250 g
Lauroglycol	2 litres

Preparation process: The pulverised active ingredient is suspended in Lauroglykol® (propylene glycol laurate, Gattefossé S.A., Saint Priest, France) and ground in a wet pulverizer to a grain size of approximately 1 to 3 μm. Portions each containing 0.419 g of the mixture are then filled into soft gelatin capsules by a capsule filling machine.

Example : Infusion solution

The compound of example 1, 2, 3 or 4 is dissolved at a concentration of 1 mg/ml in polyethylene glycol 300 (PEG 300) and filled into 2 ml vials. For infusion, this solution is diluted with 50 to 100 ml of 0.9% saline according to US Pharmacopoeia.

Detailed Description of Figures

Figure 1shows the structures of selected natural and designed epothilones. Grey boxes indicate compounds synthesized in this study.

Figure 2 is a chart illustrating the displacement of the fluorescent taxoid Flutax-2 (50 nM) from microtubule binding sites (50 nM) by competing ligands at 37 °C. The dots indicate acquired data points and the lines were generated so that they give the best fit value of the binding equilibrium constant of each competitor, assuming one-to-one binding to the same site. Ligands assayed are paclitaxel (Taxol®) (dark blue), epothilone A (**1**) (red), epothilone B (**2**) (violet), compound **3** (yellow), compound **4** (light brown), and compound **8** (green). Representative curves for selected epothilone analogues (**3**, **4**, and **8**) are presented in this figure to exemplify how the binding affinities were measured for each compound in Table 3.

Figure 3 shows the synthesis of 2-(thiomethyl)thiazole epothilone B (**3**) via Stille coupling. *Reagents and conditions*: $Pd_2(dba)_3 \cdot CHCl_3$ (0.2 equiv), CuI (2.0 equiv), $AsPh_3$ (0.8 equiv), DMF, 25 °C, 80%. dba = dibenzylideneacetone.

Figure 4 shows the retrosynthetic analysis of *trans*-cyclopropyl epothilone B analogues (**1 - 6**, **8**, **10**, **12–14**).

Figure 5 shows the construction of aldehyde **32**. *Reagents and conditions*: (a) See Nicolaou, K. C., et al. *ChemBioChem* **2001**, *2*, 69-75; Charette, A. B.; et al. *J. Am. Chem. Soc.* **1998**, *120*, 11943-11952; (b) NaH (1.5 equiv), BnBr (1.5 equiv), DMF, 0 → 25 °C, 12 h; (c) O_3, CH_2Cl_2:MeOH 4:1, –78 °C, 21 min; then $NaBH_4$ (3.0 equiv), –78 → 25 °C, 1 h, 89% for 2 steps; (d) MsCl (1.3 equiv), Et_3N (1.5 equiv), CH_2Cl_2, 25 °C , 1 h; (e) NaI (3.0 equiv), acetone, reflux, 40 min, 95% for 2 steps; (f) LDA (1.4 equiv), **25** (1.3 equiv), THF, 0 °C, 6 h; then **24**, –98 → –10 °C, 14 h, 84%; (g) MeI, 60 °C, 3 h; (h) 3 N HCl:pentane 1:1, 25 °C, 3 h, 88% for 2 steps; (i) LDA (2.4 equiv), **19** (2.3 equiv), THF, –78 °C, 1 h; then –40 °C, 0.5 h; then **17** at –78 °C, 5 min, 81%; (j) TBSOTf (2.0 equiv), 2,6-lutidine (3.0 equiv), CH_2Cl_2, –20 °C, 1 h; (k) HF·py, pyridine, THF, 25 °C, 4 h, 89% for 2 steps; (l) DMP (2.5 equiv), $NaHCO_3$ (2.5 equiv), H_2O, CH_2Cl_2, 25 °C, 1 h; (m) $NaClO_2$ (3.1 equiv), NaH_2PO_4 (2.1 equiv), 2-methyl-2-butene (74 equiv), *t*-BuOH, THF, H_2O, 25 °C, 1 h; (n) 2-(trimethylsilyl)ethanol (4.0 equiv),

EDC (1.5 equiv), 4-DMAP (0.1 equiv), DMF, 25 °C, 14 h, 74% for 3 steps; (o) 20% $Pd(OH)_2/C$, H_2 (1 atm), EtOH:EtOAc 1:1, 25 °C, 1 h; (p) DMP (2.5 equiv), $NaHCO_3$ (2.5 equiv), H_2O, CH_2Cl_2, 25 °C, 1 h, 84% for 2 steps; (q) $MeOCH_2PPh_3Cl$ (3.0 equiv), NaHMDS (2.8 equiv), THF, –40 → –10 °C, 2 h, 84%; (r) PPTS (8.0 equiv), dioxane:H_2O 9:1, 70 °C, 6 h, 82%. 4-DMAP = 4-(dimethylamino)pyridine; DME = 1,2-dimethoxy-ethane; DMP = Dess-Martin periodinane; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HF·py = hydrogen fluoride-pyridine complex; NaHMDS = sodium hexamethyldisilazide; PPTS = pyridinium para-toluenesulfonate; TMSE = 2-trimethylsilylethyl.

Figure 6 shows the construction of vinyl iodides **20c–g**. *Reagents and conditions*: (a) TrCl (1.4 equiv), 4-DMAP (1.7 equiv), DMF, 80 °C, 48 h, 100%; (b) $Pd(PPh_3)_2Cl_2$ (0.01 equiv), CuI (0.02 equiv), HCéCCH$_3$ (1 atm), DMF, *i*-Pr_2NEt, 25 °C, 3 h, **35**: 96%; (c) (i) *n*-BuLi (4.0 equiv), (*n*-$Bu_3Sn)_2$ (4.0 equiv), CuCN (2.0 equiv), MeOH, THF, –10 °C, 12 h; (ii) I_2 (1.05 equiv), CH_2Cl_2, 0 °C, 5 min, **20c**: 80% from **35**; **20d**: 67% from **36**; **20e**: 37% from **37**; **20f**: 97% from **38**; **20g**: 58% from **39**; (d) (i) HCl(g), $CHCl_3$, 0 °C, 1 h, 69%; (ii) MOMCl (1.2 equiv), NaH (1.2 equiv), THF, 0 °C, 1 h, 50%. TrCl = triphenylmethyl chloride; 4-DMAP = 4-(dimethylamino)pyridine; MOMCl = chloromethyl methyl ether.

Figure 7 is the synthesis of epothilone analogues **8–14**. *Reagents and conditions*: (a) $CrCl_2$ (10 equiv), $NiCl_2$ (0.2 equiv), 4-*t*-butylpyridine (30 equiv), 20 (3.0 equiv), DMSO, 25 °C, overnight; (b) TBAF (4.0 equiv), THF, 0 °C, 1 h; then 25 °C, 1 h; (c) Et_3N (6.0 equiv), 2,4,6-trichlorobenzoylchloride (2.4 equiv), **41** or **42**, THF, 0 °C, 1 h; then 4-DMAP (2.2 equiv), toluene, 75 °C, 3 h; (d) 20 v/v% TFA in CH_2Cl_2, 25 °C, 3 h (except **43d**); (e) Estimated by ^{1}H NMR ; (f) Deprotection of **43d**: TMSBr (10 equiv), 4Å MS, CH_2Cl_2, –30 °C, 1 h; then 20 v/v% TFA in CH_2Cl_2, 25 °C, 3 h. TBAF = tetrabutylammonium fluoride; 4-DMAP = 4-(dimethylamino)pyridine; TFA = trifluoroacetic acid; TMSBr = trimethylsilyl bromide; MS = molecular sieves.

Figure 8 illustrates a table disclosing the cytotoxicity of epothilones **1** through **14** and paclitaxel against 1A9 human ovarian carcinoma cells and β-tubulin mutant cell lines selected with paclitaxel or epothilone A. The anti-proliferative effects of the tested

compounds against the parental 1A9 and the paclitaxel- and epothilone-selected drug-resistant clones (PTX10, PTX22 and A8, respectively) were assessed in a 72 h growth inhibition assay using the SRB (sulforhodamine-B) assay (Skehan, P.; et al. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112). IC_{50} values for each compound are given in nM and represent the mean of 3-9 independent experiments Å standard error of the mean. Relative resistance (RR) is calculated as an IC_{50} value for each resistant sub-line divided by that for the parental cell line (1A9). CP = cyclopropyl; py = 5-methylpyridine side chain; pyOH = 5-hydroxymethylpyridine side chain; 5tmpy = 5-thiomethylpyridine side chain; 6tmpy = 6-thiomethylpyridine side chain; tmt = 2-thiomethyl thiazole side chain.

Figure 9 illustrates a table disclosing the tubulin polymerization potency and cytotoxicity of epothilones **1–8**, **10–14**, and paclitaxel against human epidermoid cancer cell lines. (a) The extent of porcine tubulin polymerization (TP) by 4 μM compound was quantified relative to the effect of 25 μM epothilone B (which was defined as 100%) as described (Nicolaou, K. C.; et al. *Chem. Biol.* **2000**, *7*, 593-599). (b) Drug concentration required for maximal inhibition of cell growth (IC_{50} values given in nM) was assessed after a 96 hour drug exposure by quantification of cell mass using a protein dye method as described (Meyer, T.; et al. *Int. J. Cancer* **1989**, *43*, 851-856). KB-31: epidermoid Taxol®-sensitive cells, KB-8511: epidermoid Taxol®-resistant cells (due to Pgp overexpression). Relative resistance (RR) was calculated by dividing the IC_{50} value for the resistant cell line by that of the sensitive cell line. (c) Data from ref. 3 (%TP values for Taxol®, Epo A and Epo B were 49, 69 and 90, respectively). CP = cyclopropyl; py = 5-methylpyridine side chain; pyOH = 5-hydroxymethylpyridine side chain; 5tmpy = 5-thiomethylpyridine side chain; 6tmpy = 6-thiomethylpyridine side chain; tmt = 2-thiomethyl thiazole side chain.

Figure 10 illustrates a table disclosing binding affinities of epothilone analogues to the taxoid binding site of microtubules. (a) The binding of the different ligands to the taxoid site of microtubules was measured by the displacement of a fluorescent Taxol® derivative (Flutax-2) from its binding site (Figure 2) (Díaz, J. F.; et al. *J. Biol. Chem.* **2000**, *275*, 26265-26276). The Flutax-2 displacement isotherm of each

ligand was measured at least twice with a fluorescence polarization microplate reader in a modified procedure from the previous report (Andreu, J. M.; Barasoain, I. *Biochemistry* **2001**, *40*, 11975-11984). Cross-linked stabilized microtubules which had been stored under liquid nitrogen were employed. The binding constant of the reference ligand Flutax-2 was measured by centrifugation and fluorescence anisotropy, at each temperature (Díaz, J. F.; et al. *J. Biol. Chem.* **2000**, *275*, 26265-26276). The resulting reference value was 2.2 (10^7 M^{-1} at 37 °C. (b) The equilibrium dissociation constants (Kd) are given in nM. (c) The standard binding free energy changes (DG^0_{app}) are given in kJ mol^{-1}.

Figure 11 shows a series of structures of the various designed analogs of epothilones A and B along with the structures of epothilone A and B. The design of the present epothilone library is based on the current knowledge of structure activity relationships (SAR), specifically the facts that: epothilone B (**2**) is considerably more potent than epothilone A (**1**); a thiomethyl replacement for the methyl group on the thiazole moiety enhances the potency; and a heterocycle such as pyridine or pyrimidine replacement for the thiazole side chain is needed to maintain the proper position of the nitrogen for biological activity.

Figure 12 is a scheme showing the last step in the synthesis of many of the analogs from the vinyl iodide **15** and the corresponding aromatic stannanes. A Stille-type coupling of **15** with appropriate stannanes was carried out in the presence of $PdCl_2(MeCN)_2$, CuI and $AsPh_3$ in DMF at ambient temperature, leading directly to the analogs in the indicated yields. Reagents and conditions: a. $PdCl_2(MeCN)_2$ (0.5 eq), CuI (2.0 eq), $AsPh_3$ (1.0 eq), **64a-64d**, **66a-66d**, **67-68** (2.5 eq), DMF, 25 _C, 1-3 h, 41-80%.

Figure 13 is a scheme showing the steps required to synthesize the stannanes used in the scheme in Figure 12. The thiazole compounds (**64a-64d**) were synthesized from the commercially available 2,4-dibromothiazole (**62**) by reacting the

corresponding thiol with NaH in the presence of the dibromothiazole. Coupling of the product with $Me_3SnSnMe_3$ in the presence of $Pd(PPh_3)_4$ in toluene at 100 _C gave the desired products **64a-64d**. Reagents and conditions: a) NaH (3 eq), RSH (3 eq), i-PrOH, 24 h, 70-81%; b) $(Me_3Sn)_2$ (5-10 eq), $Pd(PPh_3)_4$ (5 mol%), toluene, 100 _C, 1-3 h, 71-88%; c) *n*-BuLi (1.1 eq), ether, -78 _C, 1 h, then *n*-Bu_3SnCl (1.2 eq), -78 to 25 _C, 1h, 49-62%.

Figure 14 is scheme illustrating the synthetic route taken to build the skeleton of the cyclopropyl analogs of epothilone B. Reagents and conditions: (a) Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2001**, *123*, 9313 and Jessie, S; Kjell, U. *Tetrahedron* **1994**, *50*, 275; (b) NaH (1.5 eq), BnBr (1.2 eq), DMF, 0 to rt, 12 h, 100%; (c) O_3, CH_2Cl_2, MeOH (4:1), -78 _C, then $NaBH_4$ (3 eq), -78 _C to rt, 1 h, 83%; (d) MsCl (1.3 eq), Et_3N (1.5 eq), DCM, rt, 1 h; (e) NaI (3 eq), acetone, rt, 12 h, 91% (2 steps); (f) LDA (1.4 eq), **25** (1.3 eq), THF, 0 _C, 6 h, then **73**, -98 to -10 _C, 14 h, 87%; (g) MeI, reflux, 3 h; (h) 3N HCl:pentane (1:1), rt, 3 h, 91% (2 steps); (i) LDA (2.4 eq), **19** (2.3 eq), THF:ether (1:1), -78 _C, 1 h, then -40 _C, 30 min, then **76** at -78 _C, 5 min, 80%; (j) TBSOTf (1.5 eq), 2,6-lutidine (2 eq), DCM, -20 _C, 1 h; (k) HF•py, pyridine, THF, 0 _C, 8 h, 86% (2 steps); (l) $(COCl)_2$ (1.2 eq), DMSO (2.0 eq), DCM, -78 _C, 5 min, then **79** (1 eq), 20 min, then Et_3N (3 eq), -78 to 0 _C; (m) $NaClO_2$ (5 eq), NaH_2PO_4 (3 eq), 2-methyl-2-butene (75 eq), *t*-BuOH, THF, H_2O, rt, 1 h; (n) 2-(trimethylsilyl)ethanol (4 eq), EDC (1.5 eq), DMAP (0.1 eq), DMF, rt, 12 h, 73% (3 steps); (o) 20% $Pd(OH)_2/C$, H_2, EtOH:EtOAc (1:1), rt, 2 h, 89%; (p) $(COCl)_2$ (1.2 eq), DMSO (2.0 eq), DCM, -78 _C, 5 min, then **81** (1 eq), 20 min, then Et_3N (3 eq), -78 to 0 _C, 99%; (q) $MeOCH_2PPh_3Cl$ (3 eq), *n*-BuLi (2.8 eq), THF, 0 _C, 1 h, then **82**, -78 to 0 _C, 2 h, 79%; (r) PPTS (10 eq), dioxane:water (9:1), 70 _C, 12 h, 81%.

Figure 15 is a scheme showing the final steps used in the synthesis of cyclopropyl analogs **48** and **50**. Reagents and conditions: (a) $CrCl_2$ (10 eq), $NiCl_2$(0.2 eq), 4-*t*-BuPy (30 eq), **84a** or **84b** (3 eq), DMSO, 25 _C, 24 h; (b) TBAF (2 eq), THF, rt, 2 h;

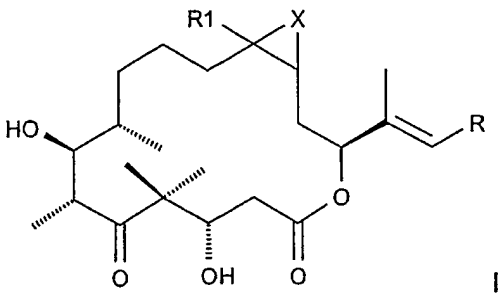
(c) Et_3N (6 eq), 2,4,6-trichlorobenzoyl chloride (2.4 eq), **85** or **88**, THF, 0 _C, 1 h, then DMAP (2.2 eq), toluene, 75 _C, 3 h; (d) 20% v/v TFA in CH_2Cl_2, rt, 3 h.

Figure 16 is a table with the cytotoxicities of epothilones **48,50** and **51-60** against human carcinoma cells and b-tubulin mutant cell lines selected with paclitaxel or epothilone A. The anti-proliferative effects of the tested compounds against the parental 1A9 and the paclitaxel- and epothilone-selected drug resistant clones (PTX10, PTX22 and A8, respectively) were assessed in a 72 h growth inhibition assay using the SRB (sulforhodamine-B) assay (Skehan, P.; et al. *J. Natl. Cancer. Inst.* **1990**, *82*, 1107–1112.). IC_{50} values for each compound are given in nM and represent the mean of 3 independent experiments Å standard error of the mean. Relative resistance (RR) is calculated as an IC_{50} value for each resistant sub-line divided by that for the parental cell line (1A9). The results for compound **3** are taken from Nicolaou, K. C.; et al. *Tetrahedron* **2002**, *58*, 6413–6432.

Figure 17 is a table with the cytotoxicities (IC_{50}'s in nM) of selected epothilones against the human epidermoid cell lines KB-3 and KB-8511. The antiproliferative effects of the tested compounds were assessed in two human epidermoid cancer cell lines, including a parent cell line (KB-31) and a Taxol™- resistant (due to Pgp-overexpression) cell line (KB-8511). The results for Epo B and **3** were taken from Nicolaou, K. C.; et al. *Tetrahedron* **2002**, *58*, 6413–6432.

What is Claimed:

1. A compound represented by formula I:

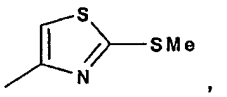


I

wherein,

when a compound of formula I is a cis- isomer X is oxygen or CH_2 and when a compound of formula I is a trans- isomer X is CH_2;

when compound of formula I is a cis- isomer and X is oxygen then R1 is methyl and R is a radical selected from the group consisting of



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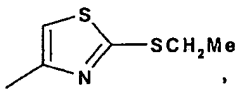

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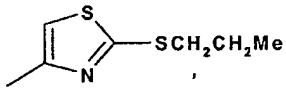

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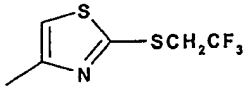

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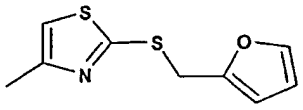

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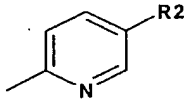

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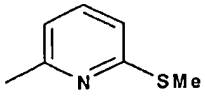
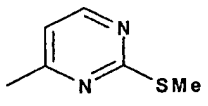
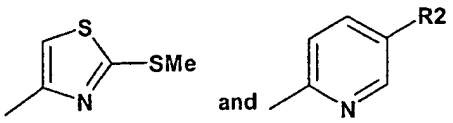


and



wherein R2 is a radical selected from the group consisting of -Me, -Cl, -Br, -SMe and $-CF_3$;

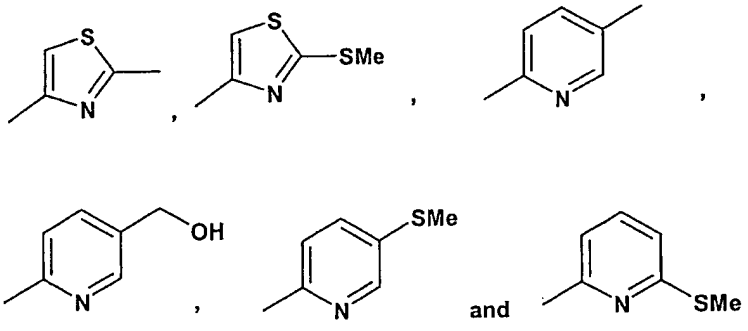
when a compound of formula I is a cis isomer and X is CH_2 then R1 is methyl and R is a radical selected from the group consisting of



wherein R2 is a radical selected from the group consisting of -Me, -Cl, -Br, -SMe and $-CF_3$;

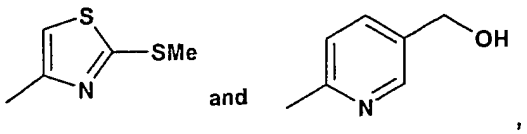
when a compound of formula I is a trans isomer and X is CH_2 then R1 is methyl or Hydrogen;

wherein, when R1 is methyl R is a radical selected from the group consisting of



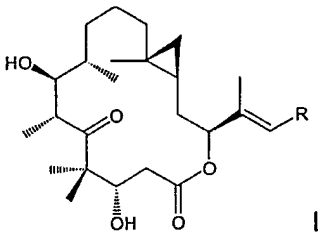
and, when R1 is hydrogen,

R is a radical selected from the group consisting of



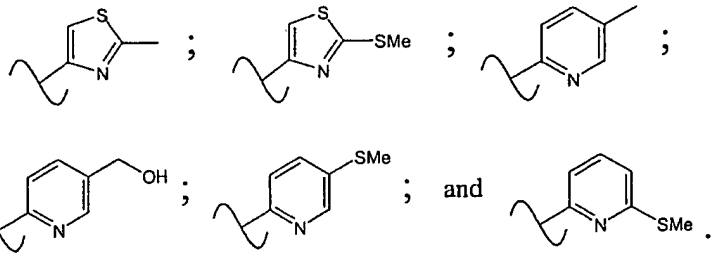
or a salt of a compound of formula I where a salt-forming group is present.

2. A compound according to claim I represented by the formula II:

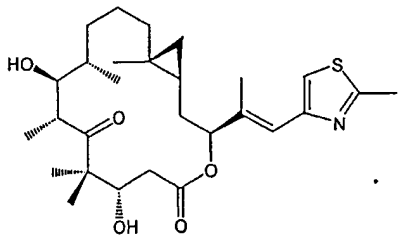


II

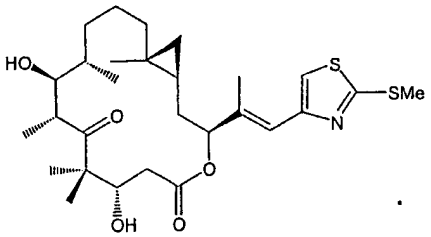
wherein R is a radical selected from the group consisting of the following structures:



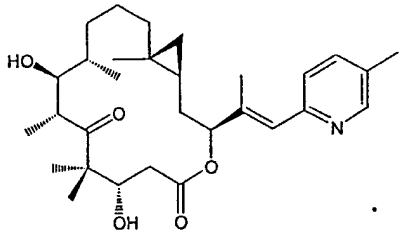
3. A compound according to claim 2 represented by the following formula:



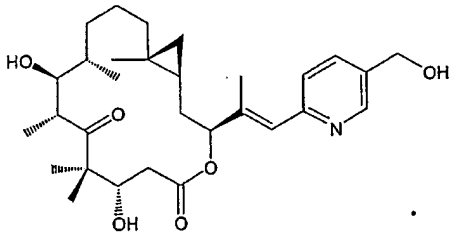
4. A compound according to claim 2 represented by the following formula:



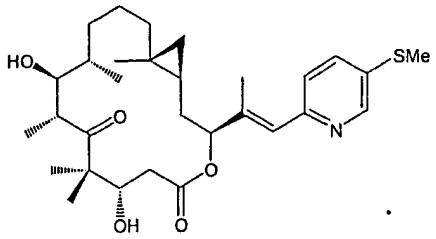
5. A compound according to claim 2 represented by the following formula:



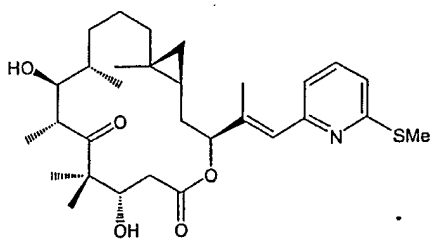
6. A compound according to claim 2 represented by the following formula:



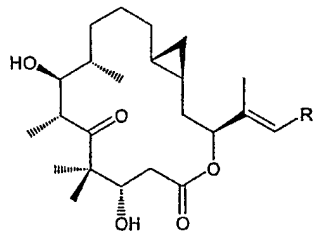
7. A compound according to claim 2 represented by the following formula:



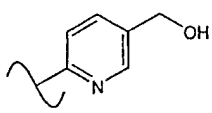
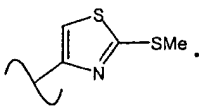
8. A compound according to claim 2 represented by the following formula:



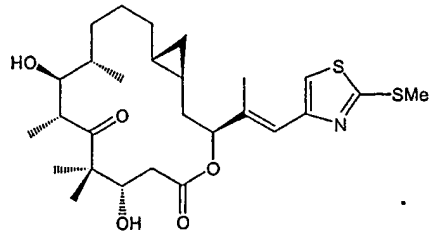
9. A compound represented by the following formula:



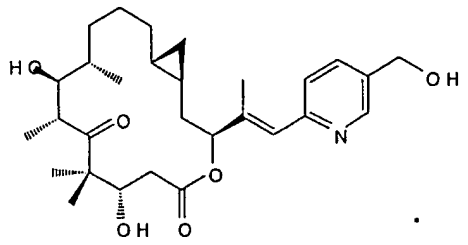
wherein R is a radical selected from the group consisting of the following structures:

 ; and  .

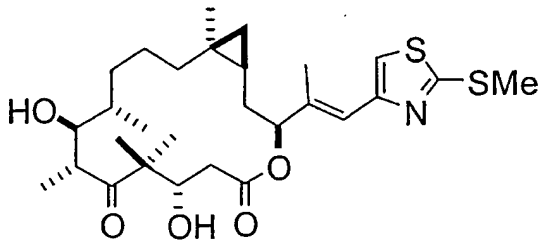
10. A compound according to claim 9 represented by the following formula:



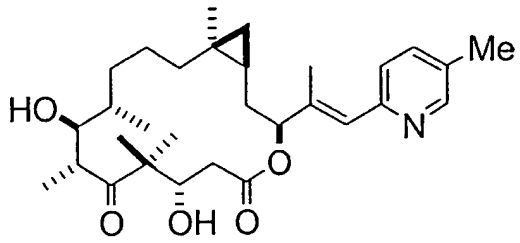
11. A compound according to claim 9 represented by the following formula:



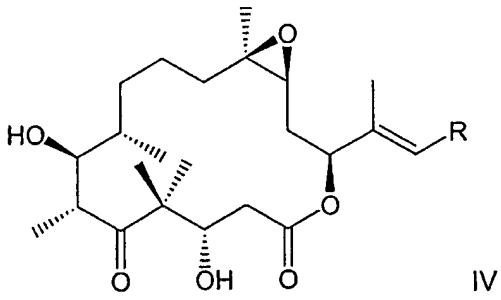
12. A compound according to claim 1 represented by the following structure:



13. A compound according to claim 1 represented by the following structure:

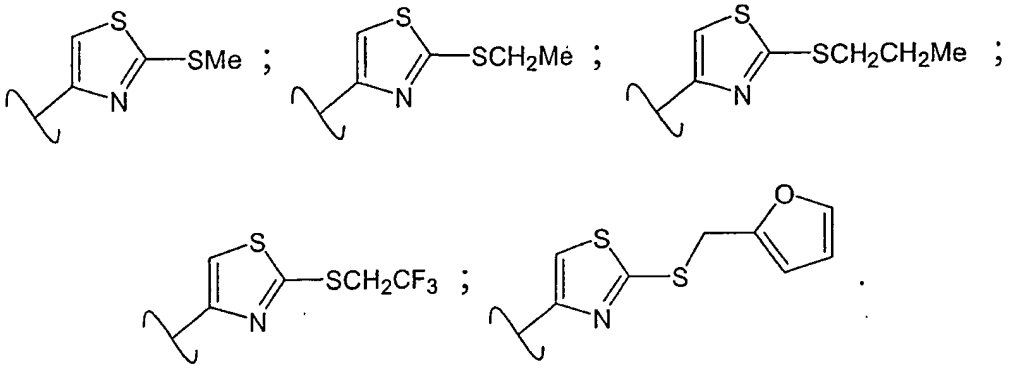


14. A compound according to claim 1 represented by formula IV:

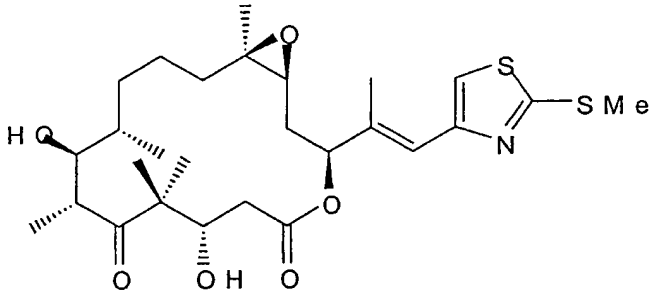


IV

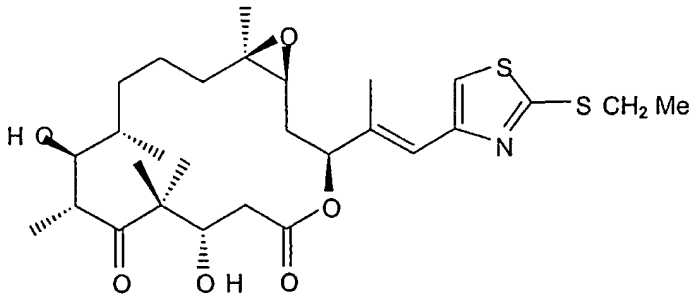
wherein R is a radical selected from the group consisting of radicals represented by the following structures:



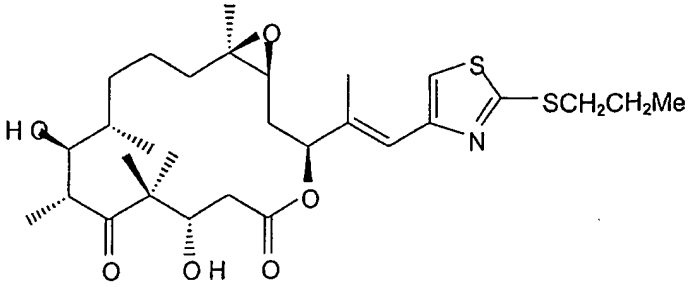
15. A compound according to claim 14 represented by the following structure:



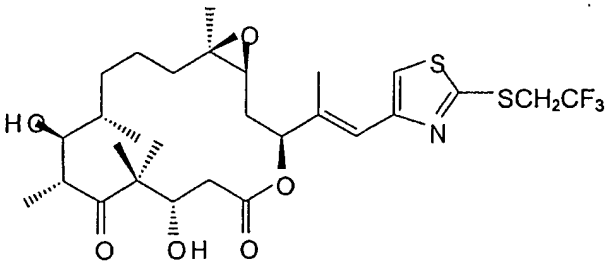
16. A compound according to claim 14 represented by the following structure:



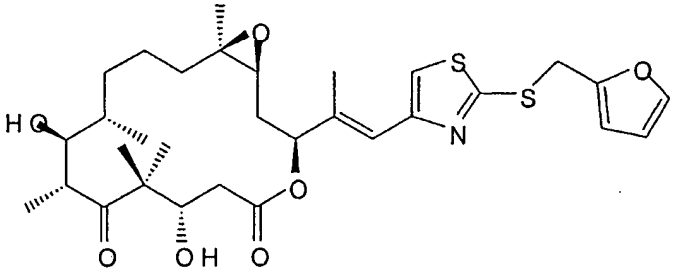
17. A compound according to claim 14 represented by the following structure:



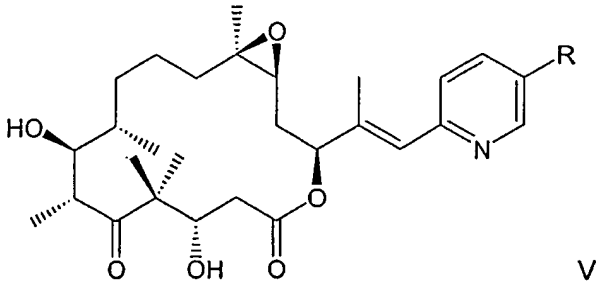
18. A compound according to claim 14 represented by the following structure:



19. A compound according to claim 14 represented by the following structure:



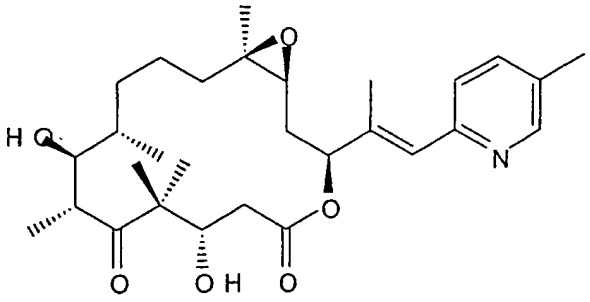
20. A compound according to claim 1 represented by the formula V:



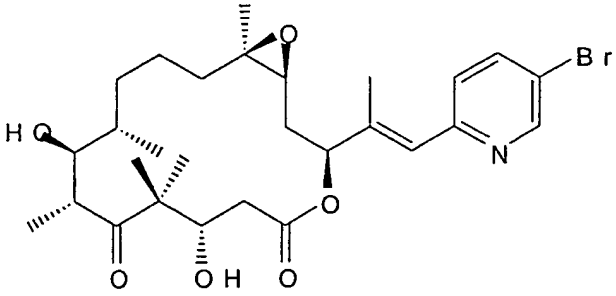
V

wherein, R is a radical selected from group consisting of -Me, -Cl, -Br, -SMe, and -CF_3.

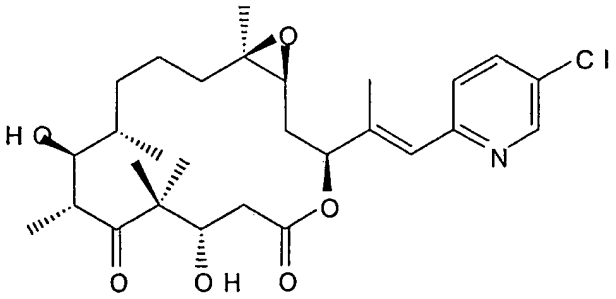
21. A compound according to claim 20 represented by the following structure:



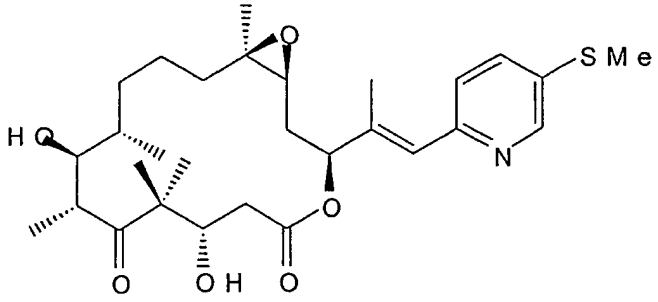
22. A compound according to claim 20 represented by the following structure



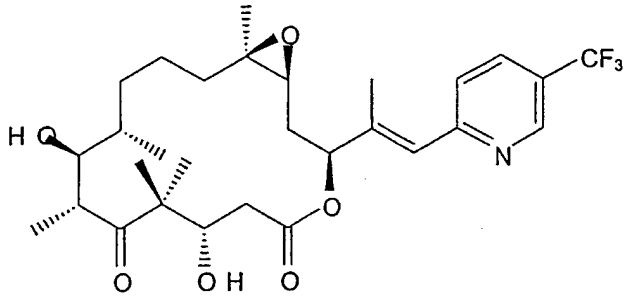
23. A compound according to claim 20 represented by the following structure



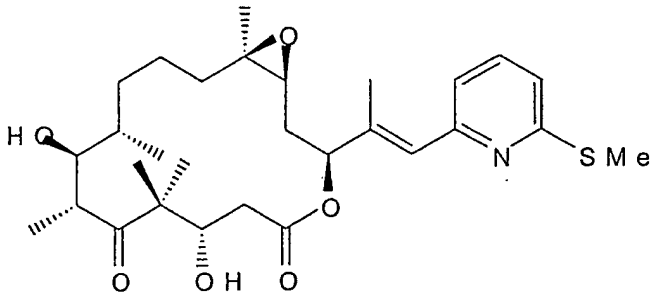
24. A compound according to claim 20 represented by the following structure



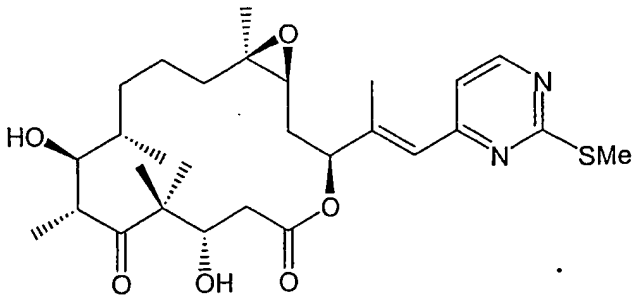
25. A compound according to claim 20 represented by the following structure



26. A compound according to claim 1 represented by the following structure:



27. A compound according to claim 1 represented by the following structure:



28. A pharmaceutical composition containing a therapeutic dose of any one or more of the compounds of claims 1-27 together with a pharmaceutically acceptable carrier for the treatment of a proliferative disease in a mammal.

29. A pharmaceutical composition according to claim 28 where the mammal is a human.

30. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and a compound according to any one of the claims 1 to 27, or a pharmaceutically acceptable acid or base addition salt thereof, where possible.

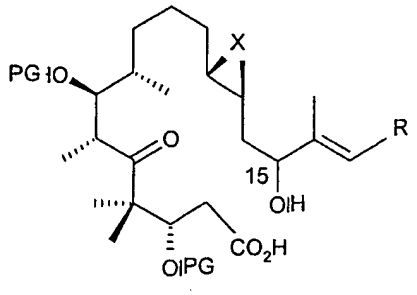
31. Use of a compound according to any one of the claims 1 to 27 for the preparation of a medicament for the treatment of a proliferative disease.

32. A compound according to any of the one claims 1 to 27 for use in a method for treatment of the human or animal body.

33. A method of treating a proliferative disease comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any one of claims 1 to 27, or a pharmaceutically acceptable acid or base addition salt thereof, where possible.

34. A process for the preparation of a compound according to any one of the claims 1-27,

wherein a compound of the formula VI

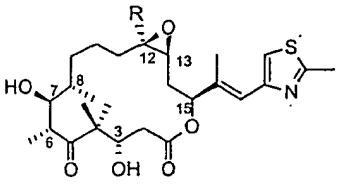


(VI)

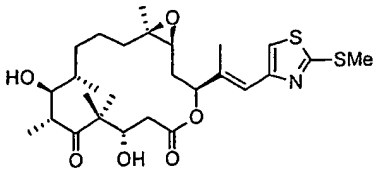
wherein X and R have any of the meanings as defined in claims 1 to 27 and PG is a protecting group for a hydroxy function,

in a first step is condensed by a esterification reaction, optionally in the presence of a catalyst,

and in a second step the protecting group is detached thus furnishing a lacton of formula I.



1: R = H : epothilone A (EpoA)
2: R = Me : epothilone B (EpoB)



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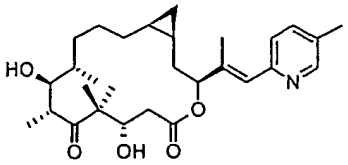

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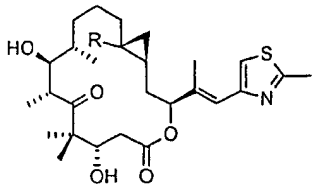
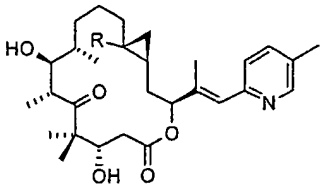
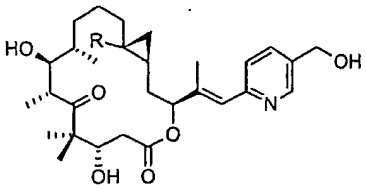


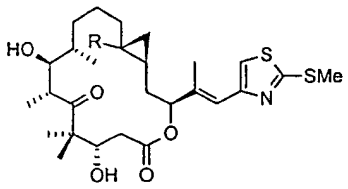
5: R = H
6: R = Me



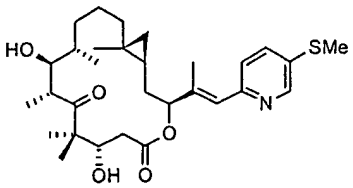
7: R = H
8: R = Me



9: R = H
10: R = Me



11: R = H
12: R = Me



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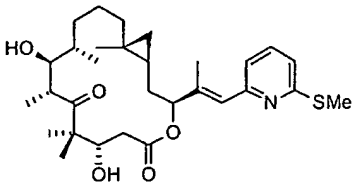


14

Figure 1

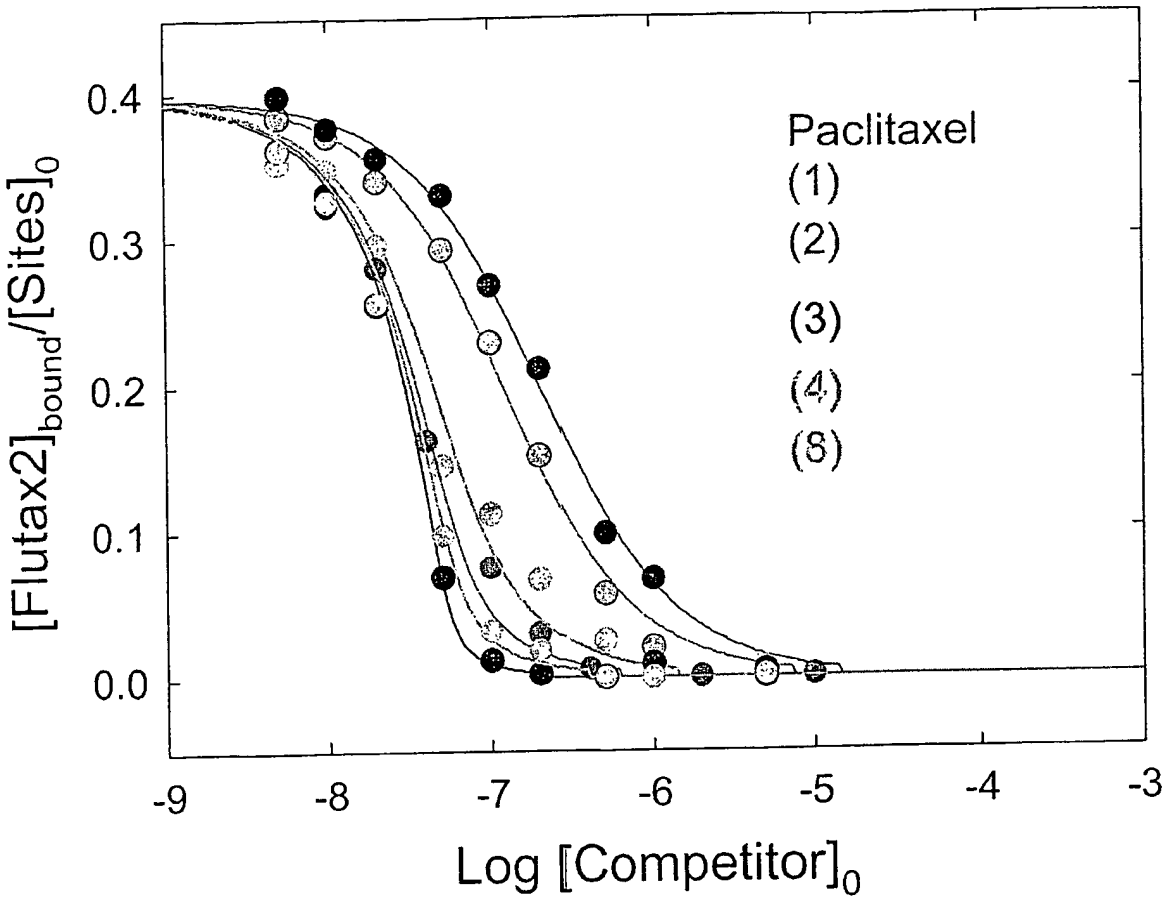
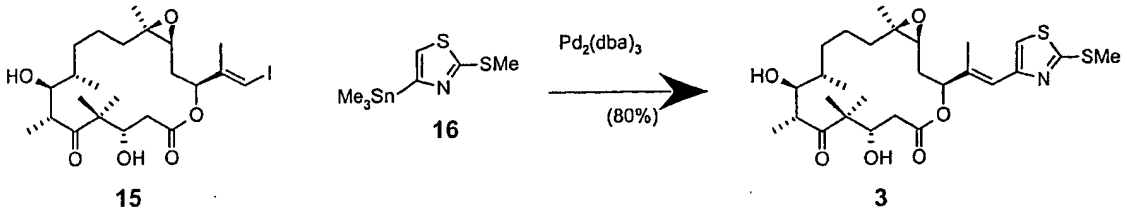

0.4
0.3
0.2
0.1
0.0
[Flutax2]bound/[Sites]0
-9
-8
-7
-6
-5
-4
-3
Log [Competitor]0
Paclitaxel
(1)
(2)
(3)
(4)
(8)

Figure 2



15 16 3

Figure 3

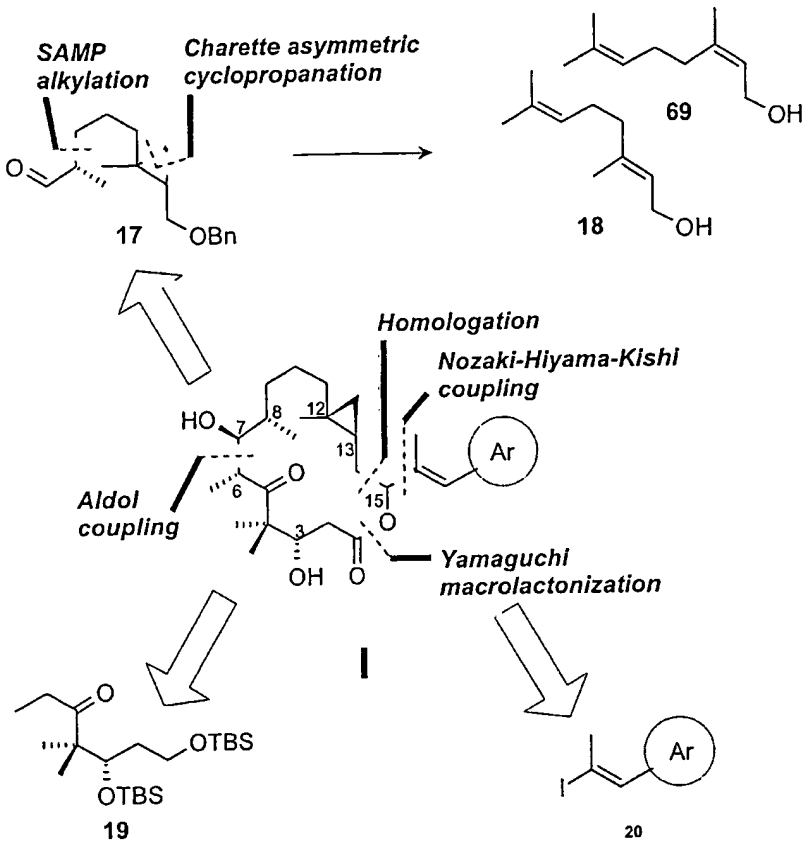

SAMP alkylation
Charette asymmetric cyclopropanation
17
OBn
69
OH
18
OH
Homologation
Nozaki-Hiyama-Kishi coupling
Aldol coupling
Yamaguchi macrolactonization
HO
7
8
12
13
6
15
3
OH
O
Ar
I
OTBS
OTBS
19
Ar
20

Figure 4

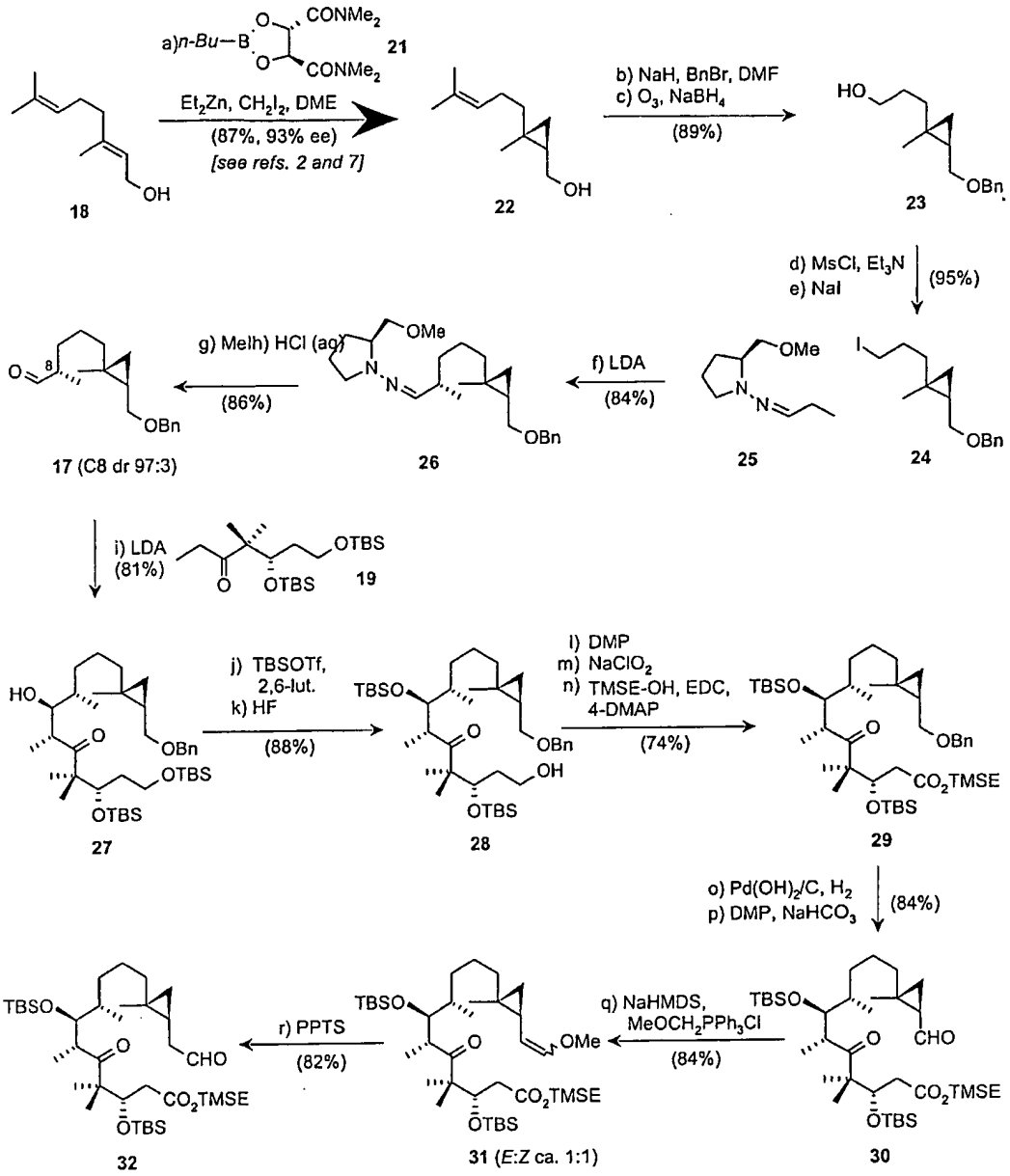

a)n-Bu—B
CONMe2
21
Et2Zn, CH2I2, DME
(87%, 93% ee)
[see refs. 2 and 7]
18
22
b) NaH, BnBr, DMF
c) O3, NaBH4
(89%)
23
d) MsCl, Et3N
e) NaI
(95%)
24
f) LDA
(84%)
25
g) MeIh) HCl (aq)
(86%)
26
17 (C8 dr 97:3)
i) LDA
(81%)
19
j) TBSOTf,
2,6-lut.
k) HF
(88%)
27
28
l) DMP
m) NaClO2
n) TMSE-OH, EDC,
4-DMAP
(74%)
29
o) Pd(OH)2/C, H2
p) DMP, NaHCO3
(84%)
30
q) NaHMDS,
MeOCH2PPh3Cl
(84%)
31 (E:Z ca. 1:1)
r) PPTS
(82%)
32

Figure 5

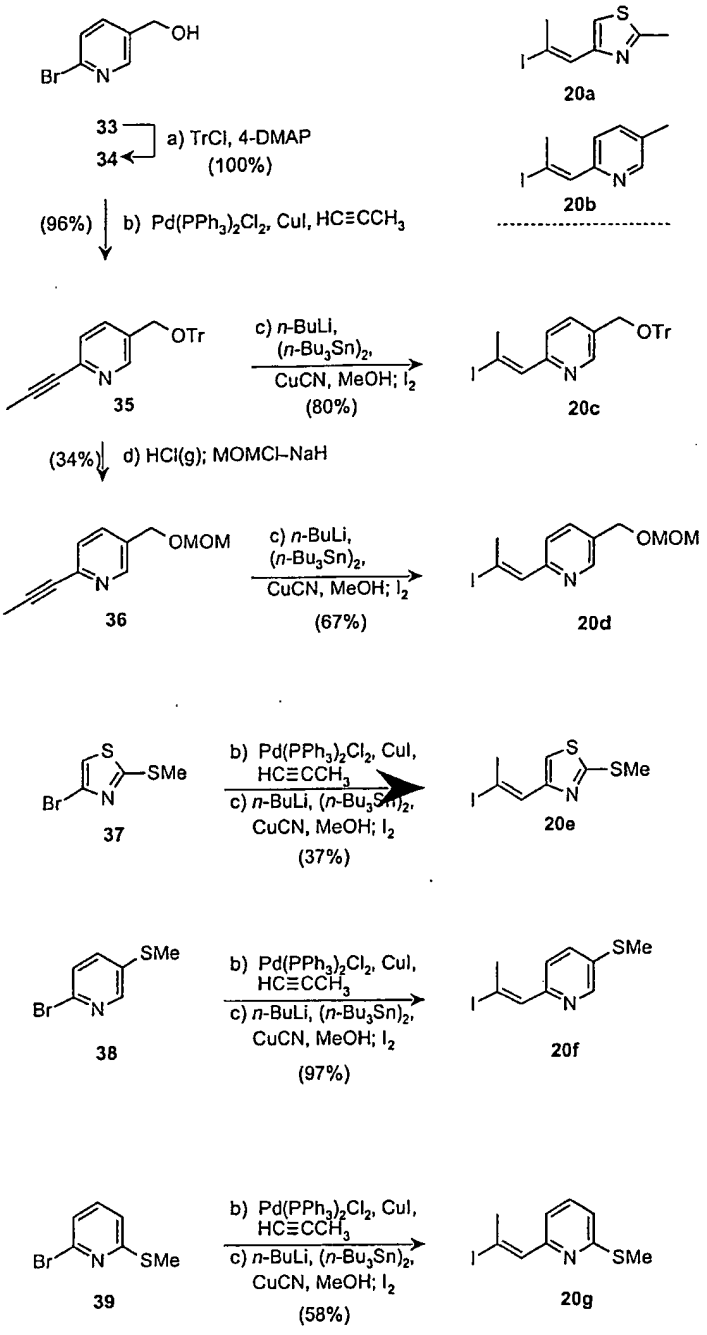
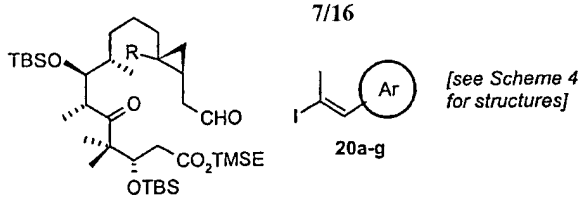
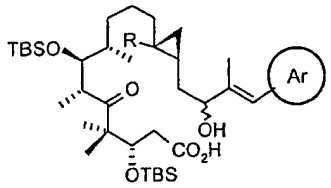

OH
Br
N
33
34
a) TrCl, 4-DMAP
(100%)
(96%)
b) Pd(PPh3)2Cl2, CuI, HC≡CCH3
OTr
35
c) n-BuLi,
(n-Bu3Sn)2,
CuCN, MeOH; I2
(80%)
20c
(34%)
d) HCl(g); MOMCl-NaH
OMOM
36
(67%)
20d
S
SMe
37
b) Pd(PPh3)2Cl2, CuI,
HC≡CCH3
c) n-BuLi, (n-Bu3Sn)2,
CuCN, MeOH; I2
(37%)
20e
38
(97%)
20f
39
(58%)
20g
20a
20b

Figure 6



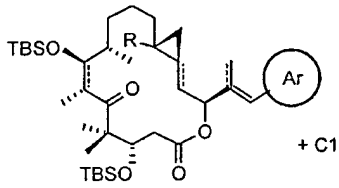
32: R = Me
40: R = H

a) $CrCl_2$, $NiCl_2$, 4-*t*-Bu-py
b) TBAF



Entry	1	2	3	4	5	6	7	8
Compound	**41a**	**41b**	**41d**	**41e**	**41f**	**41g**	**42c**	**42e**
R	Me	Me	Me	Me	Me	Me	H	H
Yield (%)	73	57	49	62	63	46	46	39

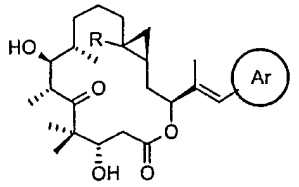
c) Et_3N, 2,4,6-trichlorobenzoyl-chloride, 4-DMAP



+ C15 epimer

Entry	1	2	3	4	5	6	7	8
Compound	**43a**	**43b**	**43d**	**43e**	**43f**	**43g**	**44c**	**44e**
R	Me	Me	Me	Me	Me	Me	H	H
Yield (%)	33	28	35	35[e]	35	37	33	47

d) 20% TFA in CH_2Cl_2[f]



Entry	1	2	3	4	5	6	7	8
Compound	**6**	**8**	**10**	**12**	**13**	**14**	**9**	**11**
R	Me	Me	Me	Me	Me	Me	H	H
Yield (%)	73	48	56	49	68	48	54	68

Figure 7

Figure 8

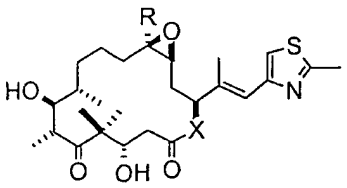
	Cell line						
	1A9	A8 (β274)		PTX10 (β270)		PTX22 (β364)	
Compound	IC_{50}	IC_{50}	RR	IC_{50}	RR	IC_{50}	RR
epothilone A (Epo A) **1**	3.1 ± 0.72	77.3 ± 9.25	24.9	29.1 ± 7.24	9.4	10.1 ± 2.10	3.3
epothilone B (Epo B) **2**	0.3 ± 0.05	6.5 ± 1.70	21.7	3.7 ± 1.83	12.3	2.1 ± 1.45	7
paclitaxel (Taxol®)	1.3 ± 0.22	11.3 ± 0.83	8.7	47.7 ± 5.01	36.7	29.4 ± 3.69	22.6
tmt-epo B **3**	0.17 ± 0.08	1.3 ± 0.65	7.6	0.26 ± 0.11	1.5	0.25 ± 0.17	1.5
cis-CP-py-epo A **4**	2.4 ± 0.99	41.6 ± 8.58	17.3	19.2 ± 9.39	8	4.2 ± 2.18	1.8
trans-CP-epo A **5**	10.1 ± 6.59	33.9 ± 5.56	3.4	17.2 ± 5.97	1.7	4.7 ± 1.68	0.5
trans-CP-epo B **6**	15	>150	>10	52	3.5	5	0.3
trans-CP-py-epo A **7**	0.6 ± 0.22	10.1 ± 2.07	16.8	5.9 ± 1.96	9.8	1.4 ± 0.51	2.3
trans-CP-py-epo B **8**	1.7 ± 0.76	27.9 ± 6.73	16.4	10.9 ± 3.52	6.4	5.6 ± 3.24	3.3
trans-CP-pyOH-epo A **9**	0.7 ± 0.16	13.0 ± 2.17	18.6	6.1 ± 1.90	8.7	1.1 ± 0.38	1.6
trans-CP-pyOH-epo B **10**	1.7 ± 1.12	13.2 ± 5.02	7.8	10.2 ± 3.75	6	2.5 ± 1.41	1.5
trans-CP-tmt-epo A **11**	1.2 ± 0.67	11.2 ± 2.30	9.3	3.2 ± 1.13	2.7	0.8 ± 0.38	0.7
trans-CP-tmt-epo B **12**	3.5 ± 1.64	28.9 ± 8.01	8.3	5.7 ± 1.96	1.6	11.5 ± 3.86	3.3
trans-CP-5tmpy-epo B **13**	14.2 ± 5.73	94 ± 5	6.6	72.0 ± 10.41	5.1	20.6 ± 9.06	1.5
trans-CP-6tmpy-epo B **14**	114	>150	>1.3	>150	>1.3	104	0.9

Figure 9

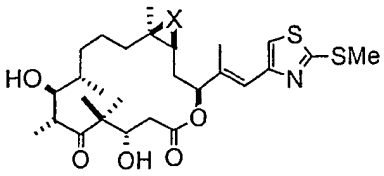
Compound	% TP[a]	*KB-31*[b]	*KB-8511*[b]	*RR*
epothilone A (Epo A) **1**	78	2.15 [c]	1.91 [c]	0.88 [c]
epothilone B (Epo B) **2**	93	0.19 [c]	0.18 [c]	0.95 [c]
paclitaxel (Taxol®)	52	2.92 [c]	626 [c]	214 [c]
Tmt-epo B **3**	99	0.11	0.07	0.61
cis-CP-py-epo A **4**	100 [c]	0.62 [c]	0.45 [c]	0.72 [c]
trans-CP-epo A **5**	100 [c]	0.97 [c]	0.64	0.66 [c]
trans-CP-epo B **6**	82	1.84	1.09	0.59
trans-CP-py-epo A **7**	94 [c]	0.84 [c]	0.68 [c]	0.81 [c]
trans-CP-py-epo B **8**	89	0.90	0.61	0.68
trans-CP-pyOH-epo B **10**	87	0.44	0.55	1.25
trans-CP-tmt-epo A **11**	93	0.66	0.32	0.48
trans-CP-tmt-epo B **12**	91	0.67	0.45	0.67
trans-CP-5tmpy-epo B **13**	88	6.88	5.28	0.77
trans-CP-6tmpy-epo B **14**	58	109	74	0.68

Figure 10

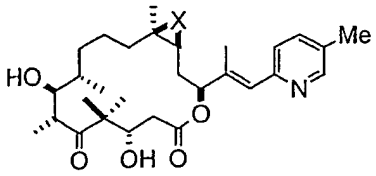
Compound	*Kd (37 °C)*[b]	ΔG^0_{app} *(37 °C)*[c]
epothilone A (Epo A) **1**	34 ± 4	-44.5 ± 0.3
epothilone B (Epo B) **2**	1.6 ± 0.1	-52.6 ± 0.5
paclitaxel (Taxol®)	93 ± 26	-42.2 ± 0.2
Tmt-epo B **3**	0.64 ± 0.24	-54.5 ± 1.2
cis-CP-py-epo A **4**	5.2 ± 0.8	-49.4 ± 0.3
trans-CP-epo A **5**	6.5 ± 0.1	-48.6 ± 0.1
trans-CP-epo B **6**	8.0 ± 1.8	-48.0 ± 0.1
trans-CP-py-epo A **7**	2.1 ± 0.4	-51.5 ± 0.2
trans-CP-py-epo B **8**	1.9 ± 0.6	-51.8 ± 0.8
trans-CP-pyOH-epo B **10**	6.0 ± 0.6	-48.9 ± 0.3
trans-CP-tmt-epo A **11**	1.6 ± 0.5	-52.2 ± 0.9
trans-CP-tmt-epo B **12**	1.8 ± 0.2	-51.8 ± 0.3
trans-CP-5tmpy-epo B **13**	1.9 ± 0.3	-51.6 ± 0.5
trans-CP-6tmpy-epo B **14**	53 ± 8	-43.1 ± 0.5



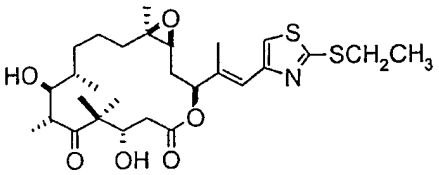
1: R = H [Epothilone A, EpoA]
2: R = Me [Epothilone B, EpoB]



3: X = O
48: X = CH2



49: X = O
50: X = CH2



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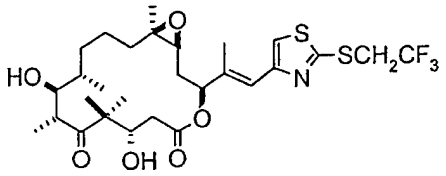

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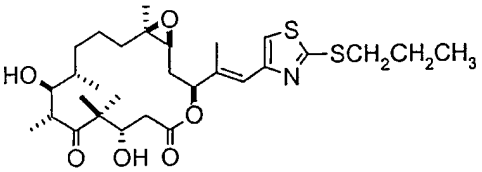

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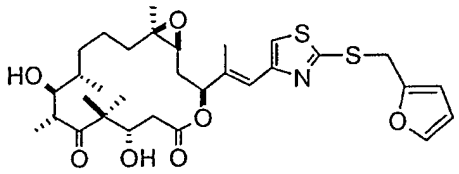

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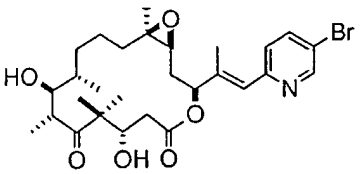

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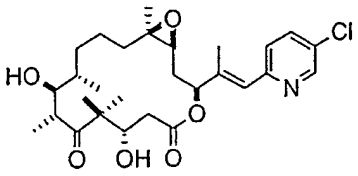

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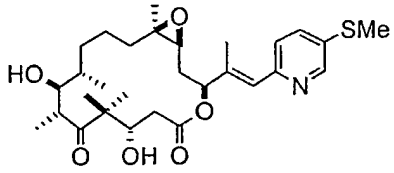

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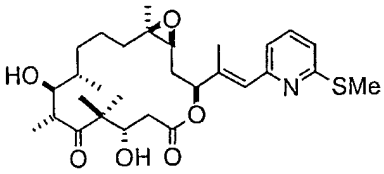

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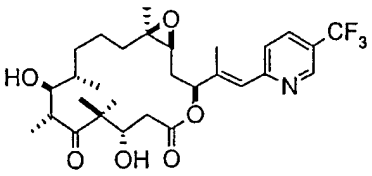
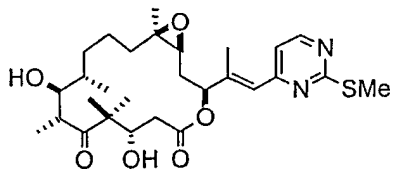


59



60

Figure 11

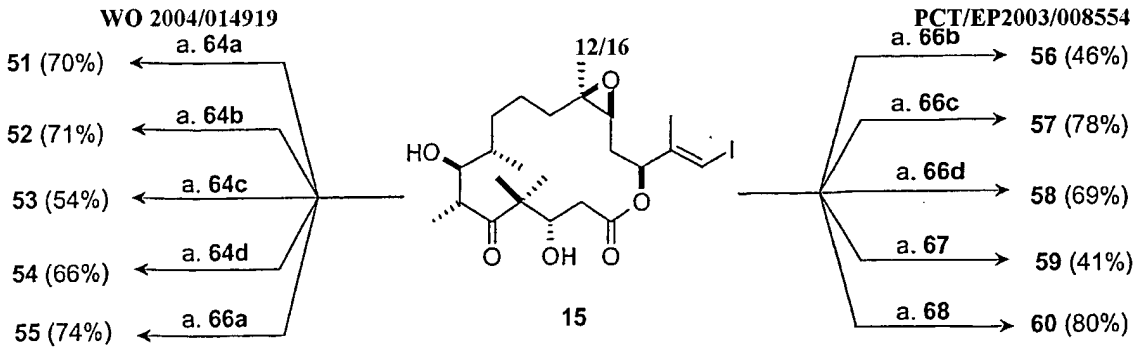
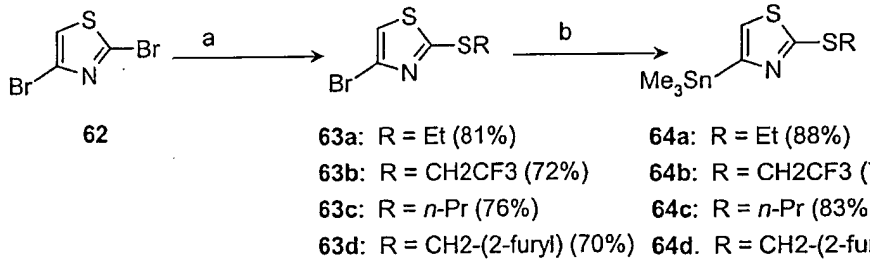


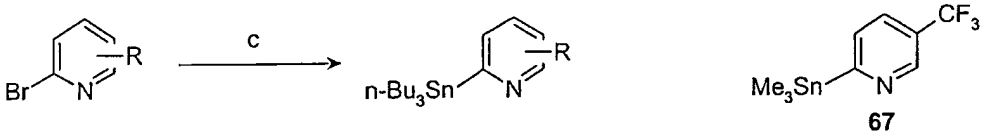
Figure 12



62

63a: R = Et (81%)
63b: R = CH2CF3 (72%)
63c: R = *n*-Pr (76%)
63d: R = CH2-(2-furyl) (70%)

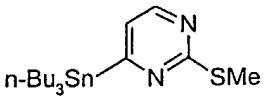
64a: R = Et (88%)
64b: R = CH2CF3 (71%)
64c: R = *n*-Pr (83%)
64d. R = CH2-(2-furyl) (69%)



65a: R = 5-Br
65b: R = 5-Cl
65c: R = 5-SMe
65d: R = 6-SMe

66a: R = 5-Br (49%)
66b: R = 5-Cl (60%)
66c: R = 5-SMe (61%)
66d: R = 6-SMe (62%)

67



68

Figure 13

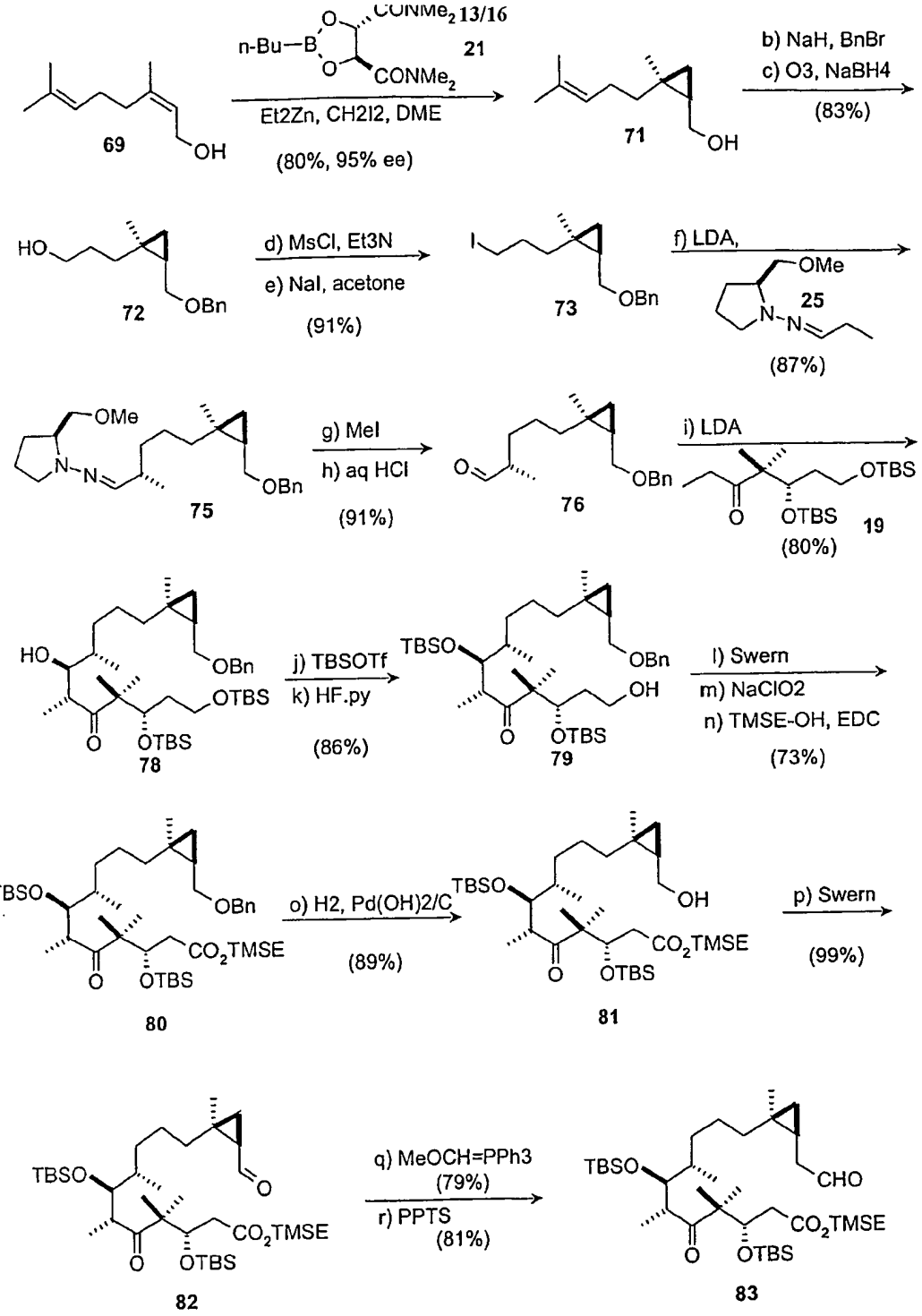

13/16
21
n-Bu—B
CONMe2
CONMe2
Et2Zn, CH2I2, DME
(80%, 95% ee)
69
OH
71
OH
b) NaH, BnBr
c) O3, NaBH4
(83%)
HO
OBn
72
d) MsCl, Et3N
e) NaI, acetone
(91%)
I
OBn
73
f) LDA,
OMe
25
N
N
(87%)
OMe
N
N
OBn
75
g) MeI
h) aq HCl
(91%)
O
OBn
76
i) LDA
OTBS
O
OTBS
19
(80%)
HO
OBn
OTBS
O
OTBS
78
j) TBSOTf
k) HF.py
(86%)
TBSO
OBn
OH
O
OTBS
79
l) Swern
m) NaClO2
n) TMSE-OH, EDC
(73%)
TBSO
OBn
CO2TMSE
O
OTBS
80
o) H2, Pd(OH)2/C
(89%)
TBSO
OH
CO2TMSE
O
OTBS
81
p) Swern
(99%)
TBSO
O
CO2TMSE
O
OTBS
82
q) MeOCH=PPh3
(79%)
r) PPTS
(81%)
TBSO
CHO
CO2TMSE
O
OTBS
83

Figure 14

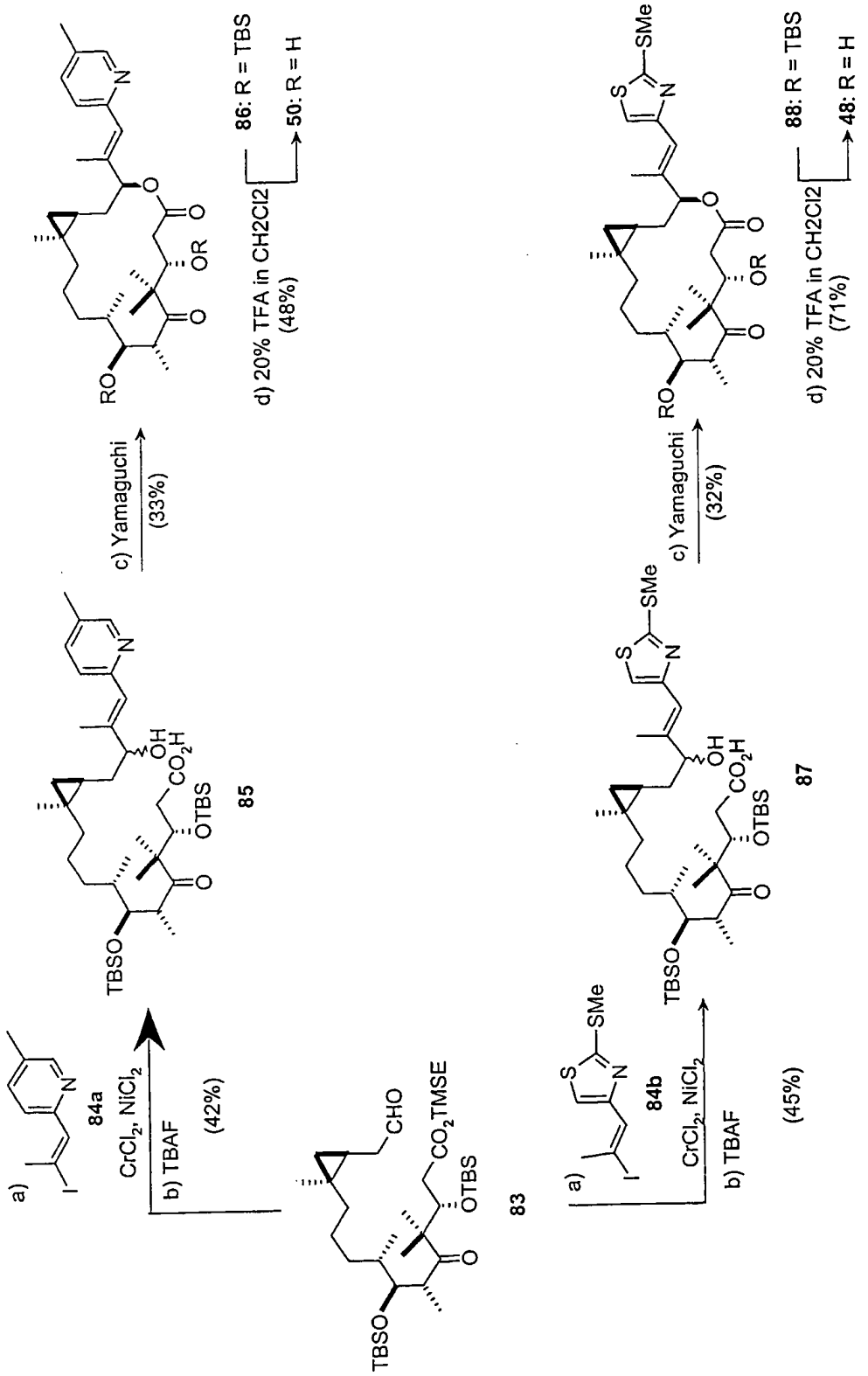

a)
84a
$CrCl_2$, $NiCl_2$
b) TBAF
(42%)
83
TBSO
CHO
CO_2TMSE
OTBS
85
OH
CO_2H
c) Yamaguchi
(33%)
86: R = TBS
d) 20% TFA in CH_2Cl_2
(48%)
50: R = H
84b
(45%)
87
SMe
(32%)
88: R = TBS
(71%)
48: R = H

Figure 15

Compound	Cell Line						
	1A9	A8		PTX10		PTX22	
	IC_{50} (nM)	IC_{50} (nM)	RR	IC_{50} (nM)	RR	IC_{50} (nM)	RR
Taxol™	3.0 ± 0.4	10.1 ± 2.9	3.3	89.7 ± 9.0	29.5	53.4 ± 26.5	17.6
Epo A	2.4 ± 0.6	91.0 ± 10.0	38.7	34.2 ± 2.0	14.5	8.7 ± 2.2	3.7
Epo B	0.6 ± 0.3	6.5 ± 0.9	10.7	3.1 ± 0.5	5.2	0.8 ± 0.5	1.3
47	0.17 ± 0.8	1.3 ± 0.65	7.6	0.26 ± 0.11	1.5	0.25 ± 0.17	1.5
48	0.1 ± 0.0	2.4 ± 1.1	23.5	0.7 ± 0.3	6.5	0.6 ± 0.5	5.9
50	0.3 ± 0.1	10.4 ± 2.4	41.4	3.3 ± 1.2	13.2	1.3 ± 1.1	5.3
52	3.5 ± 0.7	18.4 ± 1.4	5.3	16.1 ± 2.1	4.6	3.8 ± 0.3	1.1
53	4.4 ± 2.4	42.9 ± 5.1	9.7	24.7 ± 4.9	5.6	5.2 ± 0.8	1.2
54	2.1 ± 0.8	16.0 ± 5.5	7.6	9.8 ± 1.4	4.7	2.9 ± 1.3	1.4
55	0.7 ± 0.2	11.1 ± 1.0	16.6	3.9 ± 0.4	5.8	0.3 ± 0.1	0.5
56	3.2 ± 0.1	31.9 ± 3.1	10.0	16.1 ± 4.1	5.1	3.2 ± 0.3	1.0
57	0.4 ± 0.1	11.6 ± 6.7	31.7	3.9 ± 1.1	10.5	2.1 ± 1.9	5.8
58	3.3 ± 0.2	27.7 ± 3.2	8.3	12.2 ± 7.4	3.7	6.6 ± 2.6	2.0
59	4.3 ± 0.4	83.0 ± 2.0	19.2	65.3 ± 11.9	15.1	9.6 ± 1.3	2.2
60	8.6 ± 1.2	32.3 ± 2.7	3.8	42.9 ± 10.3	5.0	9.6 ± 1.0	1.1

Figure 16

	Cell Line	KB-31	KB-8511
Compound		IC_{50} (nM)	IC_{50} (nM)
Epo B		0.19	0.12
47		0.11	0.07
48		0.20	0.12
50		0.44	0.29
52		3.04	2.67
53		10.0	6.73
54		1.16	1.28
55		0.72	0.55
57		0.54	0.41
58		4.87	3.24
59		8.38	7.37
60		9.01	11.65

Figure 17

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/08554

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D493/04 C07D417/06 C07D405/06 C07D407/06 A61K31/427
A61K31/4427 A61P35/00 A61P17/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NICOLAOU K.C. ET AL: "Chemical synthesis and biological evaluation of novel epothilone B and trans-12,13-cyclopropyl epothilone B analogues" TETRAHEDRON, vol. 58, no. 32, 2002, pages 6413-6432, XP002902983 ISSN: 0040-4020 page 6418, tables 2,3 --- -/--	1-34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2003	09. 01. 2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	GERD STRANDELL

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/08554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NICOLAOU K.C. ET AL: "Design Synthesis, and Biological Properties of Highly Potent Epothilone B Analogues" ANGEW. CHEM. INT. ED., vol. 42, no. 30, 4 August 2003 (2003-08-04), pages 3515-3520, XP002262410 page, 3516, page 3518, Published Online 30 July 2003 ---	1-34
P,X	WO 03 018002 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); ALTMANN KARL-HEINZ) 6 March 2003 (2003-03-06) the whole document ---	1-34
X	WO 98 25929 A (CIBA GEIGY AG ;SARABIA FRANCISCO (ES); VALLBERG HANS (SE); NICOLAO) 18 June 1998 (1998-06-18) the whole document ---	1-34
X	DATABASE STN INTERNATIONAL ALTMANN, KARL-HEINZ ET AL: "Synthetic and semisynthetic analogs of apothilones: chemistry and biological acitivity" Database accession no. 2002:132141 XP002262419 Document no. 136:318824 abstract & ACS SYMPOSIUM SERIES (2001), 796 (ANTICANCER AGENTS), pages 112-130, ---	1-34
X	WO 99 67252 A (NOVARTIS ERFIND VERWALT GMBH ;NOVARTIS AG (CH); VALLBERG HANS (SE)) 29 December 1999 (1999-12-29) the whole document ---	1-34
X	NICOLAOU K C ET AL: "Chemical synthesis and biological properties of pyridine epothilones" CHEMISTRY & BIOLOGY, vol. 7, no. 8, 2000, pages 593-599, XP002262411 ISSN: 1074-5521 page 594, compound 4 --- -/--	1-34

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/08554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICOLAOU K C ET AL: "Chemical synthesis and biological evaluation of cis- and trans-12,13-cyclopropyl and 12,13-cyclobutyl epothilones and related pyridine side chain analogues" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 123, no. 38, 26 September 2001 (2001-09-26), pages 9313-9323, XP002262412 UNITED STATES ISSN: 0002-7863 the whole document ---	1-34
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X	NICOLAOU K.C. ET AL: "Total synthesis of 16-desmethylepothilone B, epothilone B10, epothilone F, and related side chain modified epothilone B analogues" CHEMISTRY (WEINHEIM AN DER BERGSTRASSE, vol. 6, no. 15, 4 August 2000 (2000-08-04), pages 2783-2800, XP002262415 GERMANY ISSN: 0947-6539 page 2789; examples 57A,57B,57C,57D,57E ---	1-34
X	ALTMANN KARL-HEINZ ET AL: "Synthesis and biological evaluation of highly potent analogues of epothilones B and D" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 10, no. 24, 18 December 2000 (2000-12-18), pages 2765-2768, XP002262416 OXFORD ISSN: 0960-894X page 2767, compound 14 ---	1-34

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/08554

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NICOLAOU KARL-HEINZ ET AL: "Synthesis and biological properties of C12,13-cyclopropylepothilone A and related epothilones" CHEMISTRY AND BIOLOGY, CURRENT BIOLOGY, vol. 5, no. 7, July 1998 (1998-07), pages 365-372, XP002262417 LONDON ISSN: 1074-5521 the whole document ---	1-34
X	JOHNSON J ET AL: "Synthesis, structure proof, and biological activity of epothilone cyclopropanes" ORGANIC LETTERS, vol. 2, no. 11, 1 June 2000 (2000-06-01), pages 1537-1540, XP002262418 UNITED STATES ISSN: 1523-1540 the whole document -----	1-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/08554

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 33
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 33

Claim 33 relates to a method of treatment of the human or animal body by surgery or by therapy/a diagnostic method practised on the human or animal body/Rule 39.1(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the comound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 03/08554

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9825929 A	18-06-1998	US 6441186 B1	27-08-2002
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